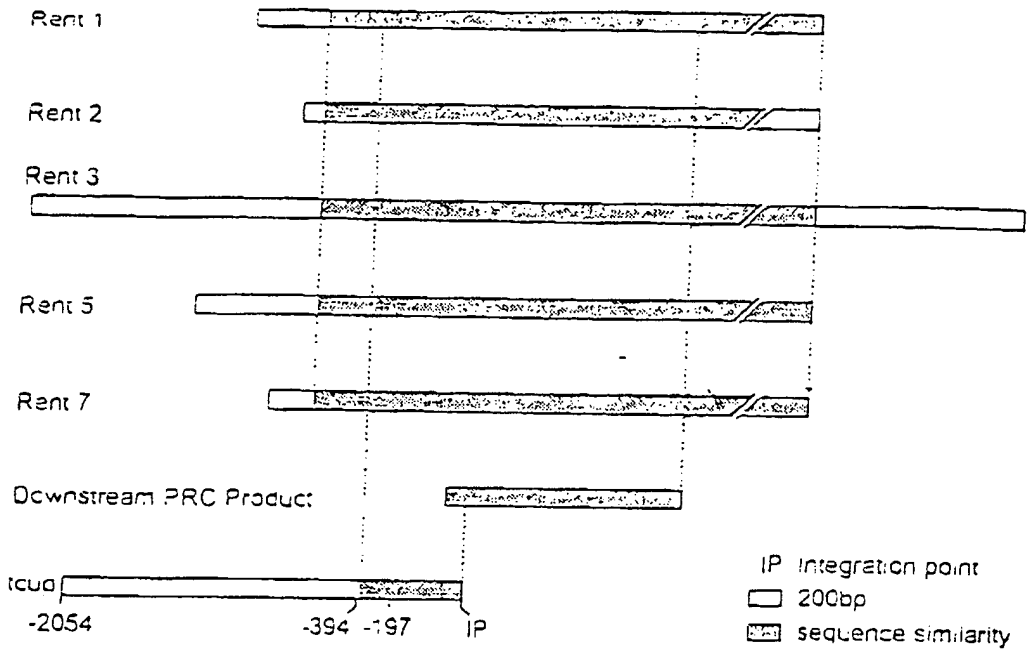

Rent 1
Rent 2
Rent 3
Rent 5
Rent 7
Downstream PRC Product
tcuo
-2054
-394
-197
IP
IP Integration point
200bp
sequence similarity

FIGURE 1A

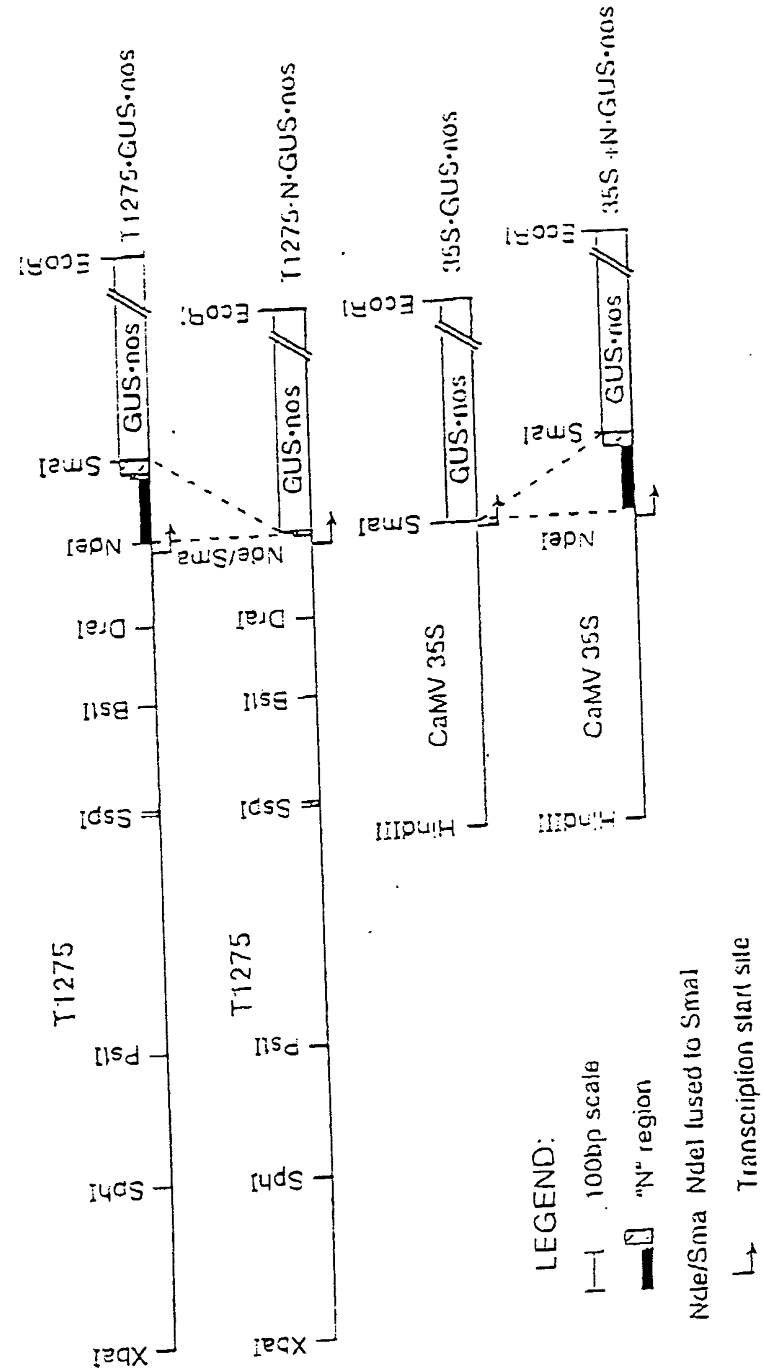

T1275·GUS·nos
T1275·N·GUS·nos
35S·GUS·nos
35S·N·GUS·nos
EcoRI
SmaI
NdeI
Nde/Sma
DraI
BslI
SspI
PstI
SphI
XbaI
HindIII
GUS·nos
CaMV 35S
T1275
LEGEND:
100bp scale
"N" region
Nde/Sma NdeI fused to SmaI
Transcription start site

FIGURE 1B

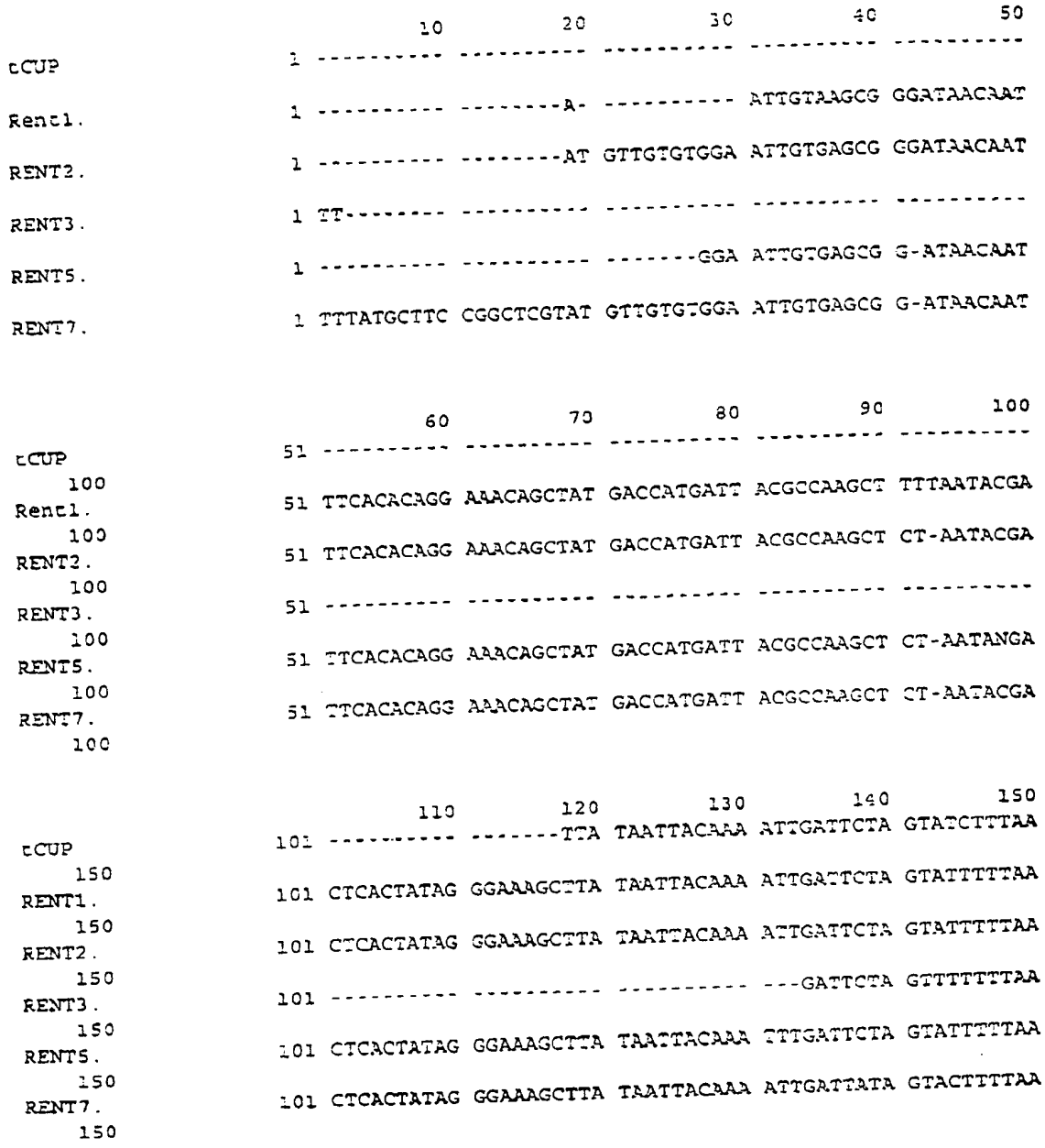

10 20 30 40 50
tCUP 1 ---------- ---------- ---------- ---------- ----------
Rent1. 1 ---------- --------A- ---------- ATTGTAAGCG GGATAACAAT
RENT2. 1 ---------- --------AT GTTGTGTGGA ATTGTGAGCG GGATAACAAT
RENT3. 1 TT-------- ---------- ---------- ---------- ----------
RENT5. 1 ---------- ---------- -------GGA ATTGTGAGCG G-ATAACAAT
RENT7. 1 TTTATGCTTC CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG G-ATAACAAT
60 70 80 90 100
tCUP 51 ---------- ---------- ---------- ---------- ----------
100
Rent1. 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT TTTAATACGA
100
RENT2. 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATACGA
100
RENT3. 51 ---------- ---------- ---------- ---------- ----------
100
RENT5. 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATANGA
100
RENT7. 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATACGA
100
110 120 130 140 150
tCUP 101 ---------- --------TTA TAATTACAAA ATTGATTCTA GTATCTTTAA
150
RENT1. 101 CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTAA
150
RENT2. 101 CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTAA
150
RENT3. 101 ---------- ---------- ---------- ---GATTCTA GTTTTTTTAA
150
RENT5. 101 CTCACTATAG GGAAAGCTTA TAATTACAAA TTTGATTCTA GTATTTTTAA
150
RENT7. 101 CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTATA GTACTTTTAA
150

FIGURE 1C1

```
                        160         170         180         190         200
cCUP          151 TTTAATGCTT ATACATTATT AATTAATTTA GTACTTTCAA TTTGTTTTCA
    200
RENT1.        151 TTTAATATTT TTACATTATT AATTAATTTA GAAGTTTTAA TTTTTTTTCA
    200
RENT2.        151 TTTAATATTT ATACATTATT AATTAACTTA GTACTTTCAA TTCGTTTTCA
    200
RENT3.        151 TTTAATATTT ATACATTATT AATTAATTTA GTTCTTTCAA TTTGTTTTCA
    200
RENT5.        151 TTTAATATTT ATACATTATT AATTAATTTA GTACTTTCAA TTTGTTTTCA
    200
RENT7.        151 TTTAATATTT ATACATTATT AATTAATTTA GCACTTTCAA TTTATTTTCA
    200

                        210         220         230         240         250
cCUP          201 GAAATTATTT TACTATTTTT TATAAAATAA AAGGGAGAAA ATGGCTATTT
    250
RENT1.        201 GAAATCATTT TACTATTTTT -ATAAAAACA AAAGGGAAAA GTGGTTATTT
    250
RENT2.        201 AAAATTATTT TACTATTTTT TGTAAAATAA AAGGGAGAAA ATGGCTATTT
    250
RENT3.        201 GAAATTATTT TACTATTTTT TATAAAATAA AAGGGAGAAA ATGGCTATTT
    250
RENT5.        201 GAAATCATTT TACTATGGTT TATAAAATAA AAGGGAGAAA ATGGCTATTT
    250
RENT7.        201 GAAACCATTT TACTATTTTT TATAAAATAA AAGGGACAAA ATGGCTATTT
    250

                        260         270         280         290         300
cCUP          251 AAATACTAGC -CTATTTTAT TTCAATTTTA GCTTAAAATC AG-CCCCAAT
    300
RENT1.        251 AAATACTAGC CCTATTTCAT TTCAATTATA GCCTAAAATC AGCCCC-AAT
    300
RENT2.        251 AAATACTAGC CCTATTTTAT TTCAATTTTA GCCTAAAATC AGCCCCCAAT
    300
RENT3.        251 AAATACCAGC CCTATTTTAT TTCAATTTTA ACCTAAAATC AGCCCC-AGT
    300
RENT5.        251 AAATACTAGC CCTATTTTAT TTCAATTTTA GCCTAAAATC AGCCCC-AAT
    300
RENT7.        251 AAATACCAAC ACTATTTTAT TTCAATTTTA GCCTAAAATC AAACCC-AAT
    300
```

FIGURE 1C2

```
                                    310        320        330        340        350
301 TAGCCCCAAT TTCAAATTCA AATGGTCCAG CCCAATTCCT AAA-TAACCC

tCUP
    350
301 TAACCCCAAT TCCAAATTCA AACGGGCCAG CCCAATTCCT AAAATGACCC
RENT1.
    350
301 TAACCCCAAT TTCAAATTCA AATGGGACAG CCCAATTCCT AAAATAACCC
RENT2.
    350
301 TAGCCCC--- ---------A AACGGCCCAT CCCAATTCCT AAAATAACTC
RENT3.
    350
301 TAACCCCTAT TTCAAATTCA AACGGGCTAG CCCAGTTCCT AAAATAACCC
RENT5.
    350
301 TAACCCC--- ---------A AACGGGCCAG CCCAATTCCT AAAACAACCC
RENT7.
    350

                                360        370        380        390        400
351 ACCCCTAACC C--------- ----GCCCGG TTTCCCCTTT TGATCCAGGC

tCUP
    400
351 GCTCCTAACC CGCTTTTCCA ACCCGCCCGG TTTCCCCTTT TGATCCAGGC
RENT1.
    400
351 GCCCCTAACC CTCTTATCCA ACCCACCCGA TTTCCCCTTT TGATCCAGGT
RENT2.
    400
351 GCCCCTAACC CGCTTATCCA ACCCGCCCGG TTCCC-CTTT TGATCCAGGC
RENT3.
    400
351 TCCCCTAACC CGCTTATCCA ACCCGCCCTG TTTCCCCTTT TGATCCAGGC
RENT5.
    400
351 GCCCCTAACC CGCTTATCCA ACCCGCCCGA TTTCCTCTTT TGATCCAGGC
RENT7.
    400

                                410        420        430        440        450
401 CGTTGATCAT TTTGATCAAC GCCCAGAATT TCCCCTTTTC CTTTTTTAAT

tCUP
    450
401 TGTTGATCAT TTTGATCAAC GGCCAGAATT TCCCCTTTCC --TTTTTAAT
RENT1.
    450
401 TGTTGATCAT TTTGATCAAC GACCAGAATT TCCCCCTTCC TGTTTTTAAT
RENT2.
    450
401 CGTTGATCAT TTTGATCAAC GACCAGAATT TCCCCTTTCC -TTTTTTAAT
RENT3.
    450
401 CGTTGATCAT TTTGATCAAC GACCAAAATT TCCCCTTT-C CTTTTTTAAT
RENT5.
    450
401 CGTTGATCAT TTTGATCAAC GGCCAGAATT TCCCCTTTCC -TTTTTTCAT
RENT7.
    450
```

FIGURE 1C3

```
                                460        470        480        490        500
451 TCCCAAACAC C-CCTAACTC TATCCCATTT CTCACCAACC GCCACATATG
tCUP
    500
451 TCCCAAACAC CCCCCAACCT TATCCCGTTT CTCACCAACC GCCAGATCT-
RENT1.
    500
451 TCCCAAACAC CCCCCAACCC TATCCCATTT CTCACCAACC GCCAGATCT-
RENT2.
    500
451 TCCCAAACAC CGCC-AAACC TATCCCATTT CTCACCAACC GCCAGATCTA
RENT3.
    500
451 TCCCAAACAC CCCC-AACCC TATCCCATTT CTCACCAACC GCCAGATCT-
RENT5.
    500
451 TCCCAAACAC CCCC-AAACC TATCCCATTT CTCACCAACC GCCAGATCTA
RENT7.
    500

                              510        520        530        540        550
501 AATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCTAA CCCTAGCAGC
tCUP
    550
501 -ATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCTAA CCCTAGCAGC
RENT1.
    550
501 -ATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCTAA CCCTAGCAGC
RENT2.
    550
501 T--CCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCTAA CCCTAGCAGC
RENT3.
    550
501 -ATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCTAA CCCTAGCAGC
RENT5.
    550
501 T--CCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCTAA CCCTAGCAGC
RENT7.
    550

                              560        570        580        590        600
551 CTCTCATCAT CCTCACCTCA AAACCCACCG GAATACATGG CTTCTCAAGC
tCUP
    600
551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAG-
RENT1.
    600
551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAG-
RENT2.
    600
551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAGG
RENT3.
    600
551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAG-
RENT5.
    600
551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAGG
RENT7.
    600
```

FIGURE 1C4

```
                                  610        620        630        640        650
601 CGTGGAAACC TTATACTCAC CTCCCTTTGC TCTTACAGTA CTC-GGCCGT

tCUP
    650
RENT1.
    650
RENT2.
    650
RENT3.
    650
RENT5.
    650
RENT7.
    650

601 ---GATCCCC GGGTGGTCAG TCCCTTATG- --TTAC---- --------GT

601 ----GA---- ---------- -TCCCCG--- ------GGTG GTC-AGTCCC

601 ATC----CCC GGGTGGTCAG TCCCTTATGT NA------CG NCCTAAATGN

601 ---GATCCCC GGGTGGTCAG TCCCTTATG- --TTACG--- ----------

601 ATC----CCC GGGTGGTCAG TCCCTTATGT TA------CG TCCTN-----

                                  660        670        680        690        700

651 CGACCGCGGT ACCCGGG... .......... .......... ..........

tCUP
    700
RENT1.
    700
RENT2.
    700
RENT3.
    700
RENT5.
    700
RENT7.
    700

651 C---------- --CTNAA... .......... .......... ..........

651 TTAT-GTG-- ---CGTC... .......... .......... ..........

651 CCGNCCTGNN NNNNN-C... .......... .......... ..........

651 ---------- TCC----... .......... .......... ..........

651 ---------- --------... .......... .......... ..........
```

FIGURE 1C5

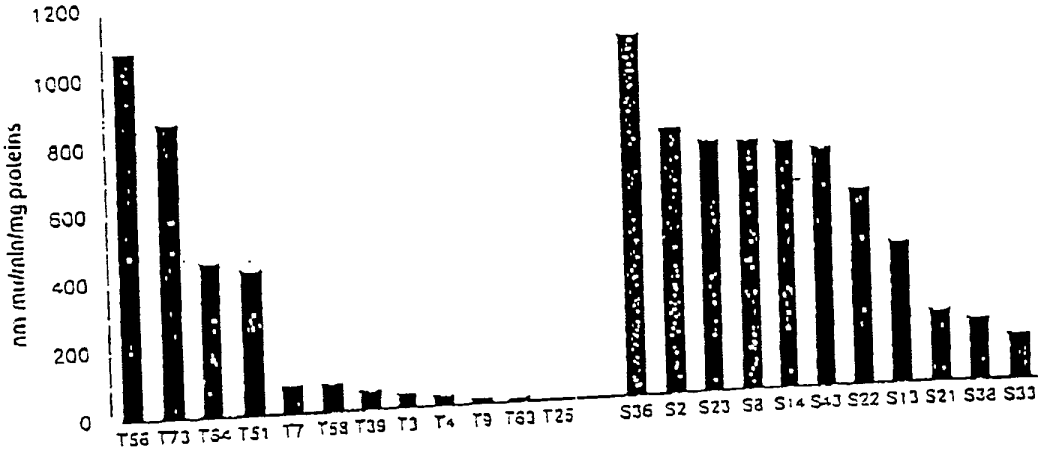


FIGURE 2A

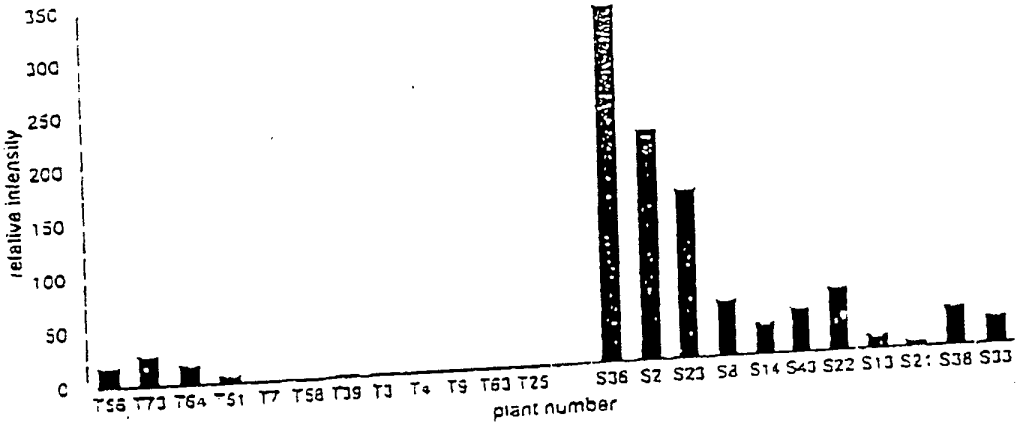


FIGURE 2B

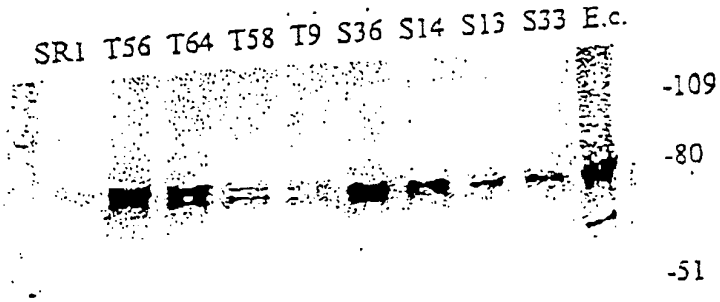


FIGURE 2C

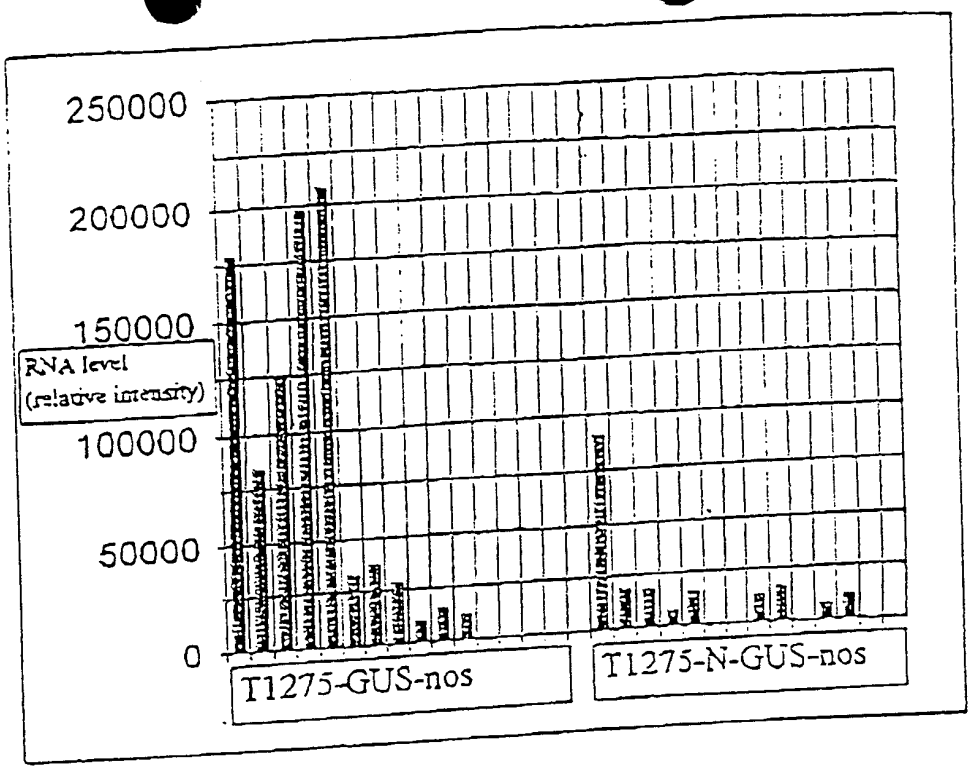


FIGURE 3A

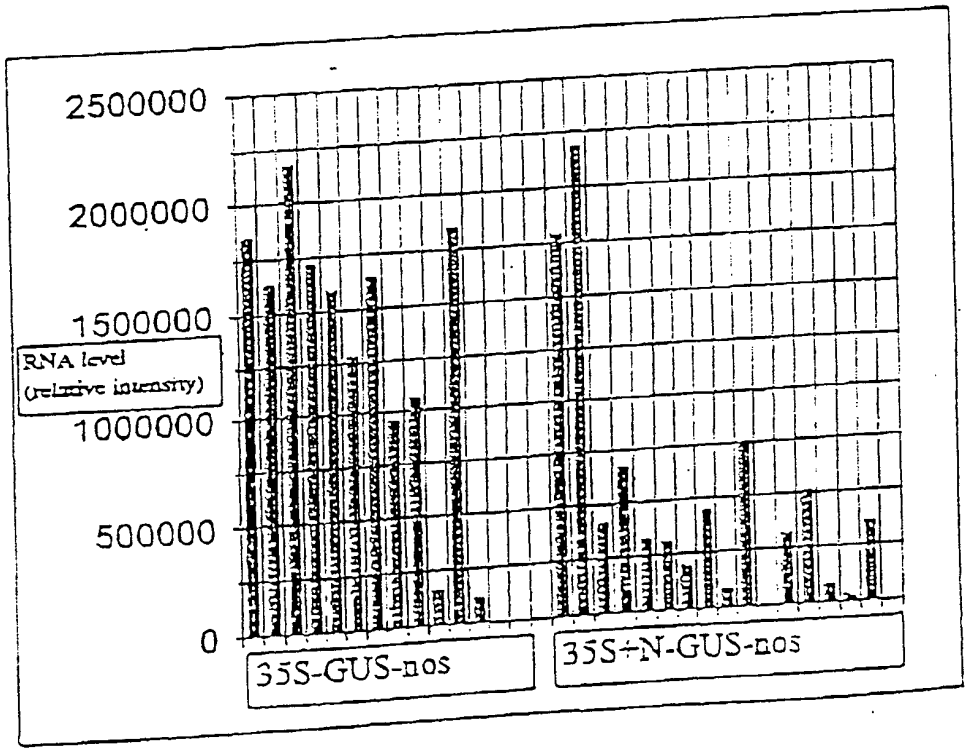


FIGURE 3B

GUS Specific Activity : Relative RNA Level
0
5
10
15
20
25
30
35
1CUP-GUS-nos
1CUP-N-GUS-nos
35S-GUS-nos
35S+N-GUS-nos

FIGURE 3C

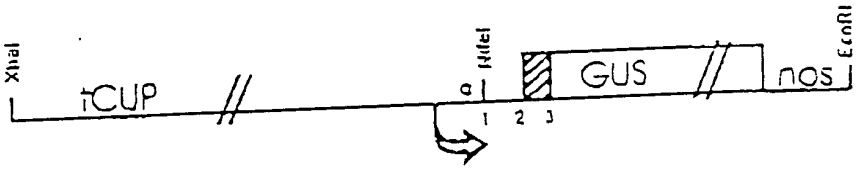
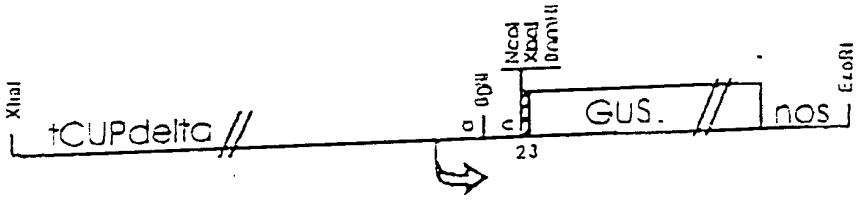


FIGURE 4A



a NdeI position +30 ACA TAT GAA

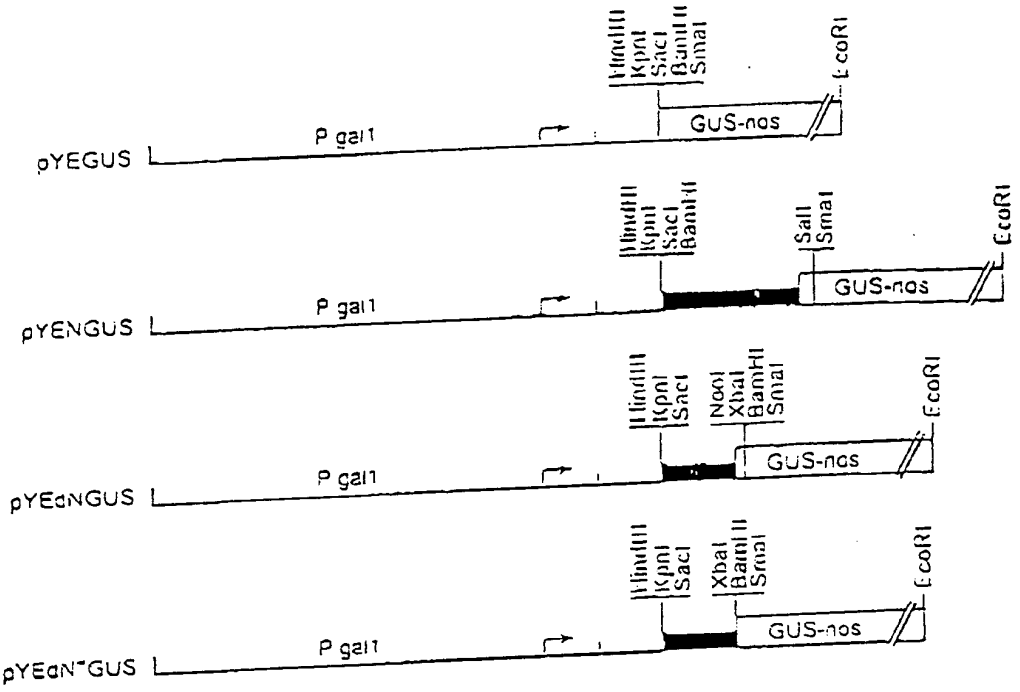
b BglII position +29 ACA GAT CT

c NcoI position 0 CC ACC ATG GCC TCT AGA GGA TCC CCG GGT GGT CAG TCC CTT ATG

tCUP initiation site GAA TAC ATG G /.. tCUP leader .../ CCG GGT GGT CAG TCC CTT ATG

Kozak consensus CC ACC ATG G

FIGURE 4B



LEGEND:

- Vector sequence
- N, dN, or dN⁻
- UidA reporter gene
- Start of transcription
- 50bp

FIGURE 5A

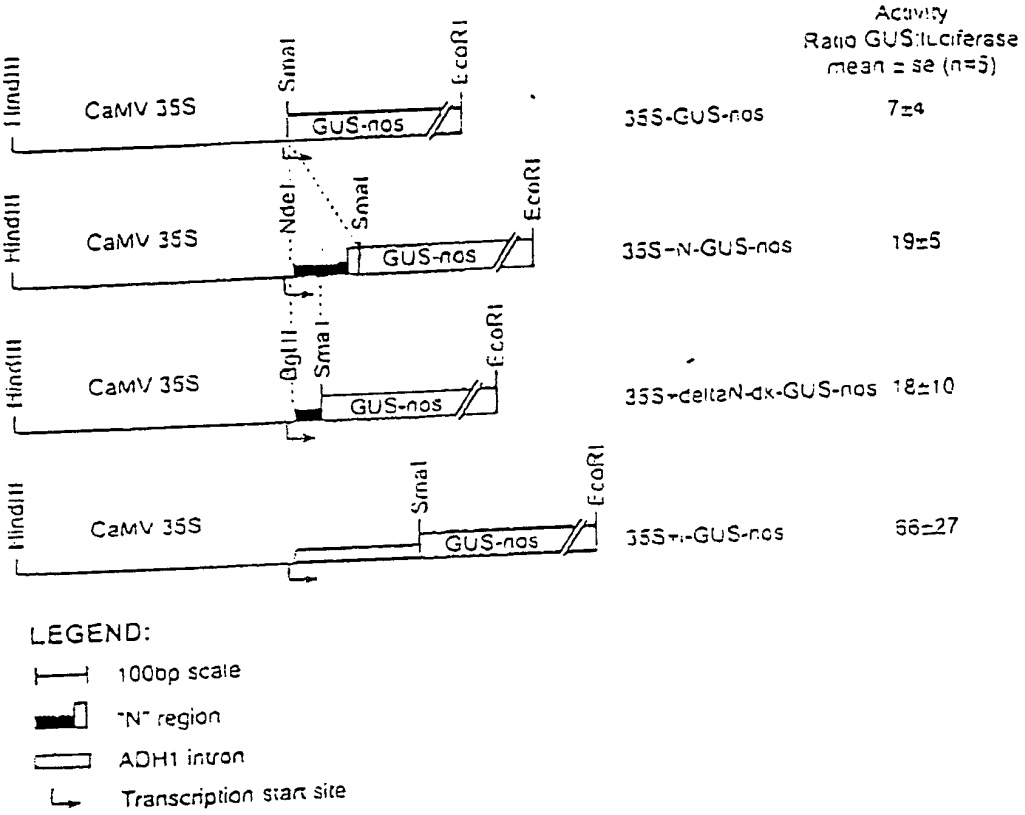

Activity
Ratio GUS:luciferase
mean ± se (n=5)
HindIII
CaMV 35S
SmaI
EcoRI
GUS-nos
35S-GUS-nos
7±4
NdeI
35S-N-GUS-nos
19±5
BglII
35S-deltaN-dx-GUS-nos
18±10
35S-i-GUS-nos
66±27
LEGEND:
100bp scale
"N" region
ADH1 intron
Transcription start site

FIGURE 5B

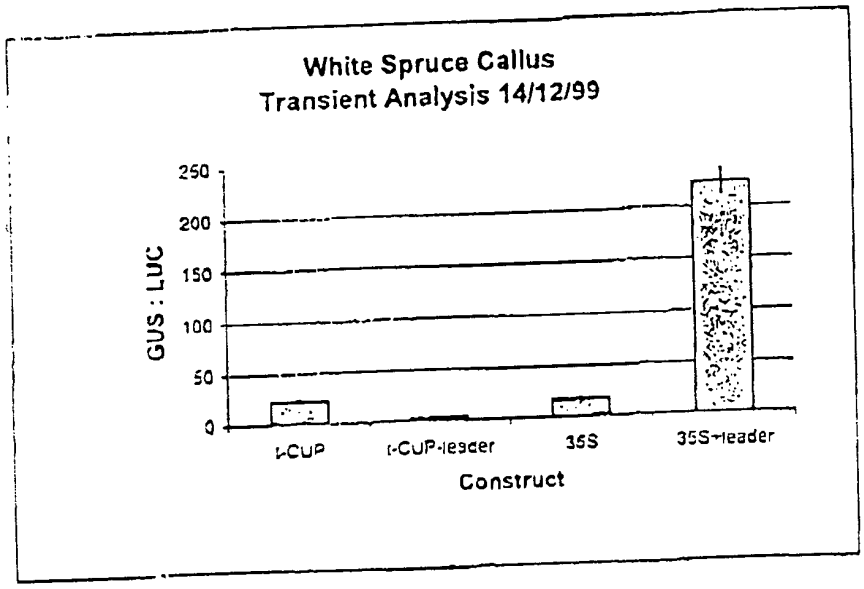

White Spruce Callus
Transient Analysis 14/12/99
GUS : LUC
250
200
150
100
50
0
t-CUP
t-CUP-leader
35S
35S-leader
Construct

FIGURE 5C

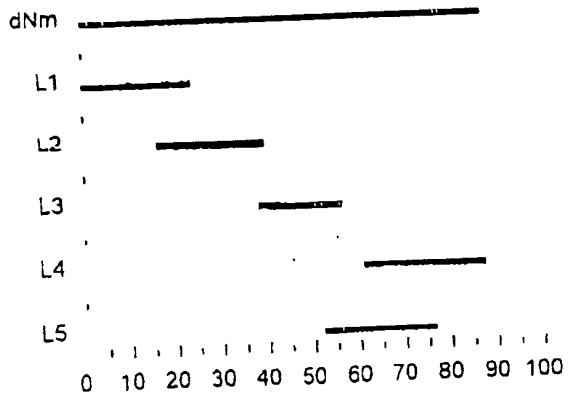


FIGURE 6A

Linker 1: GGATCTATCCTCTTATCTCTCAA

Linker 2: ATCTCTCAAACTCTCTCGAACCTT

Linker 3: TTCCCCTAACCCTAGCAG

Linker 4: ATCATCCTCACCTCAAAACCCACC

Linker 5: AGCCTCTCATCATCCTCACCTCAA

FIGURE 6B

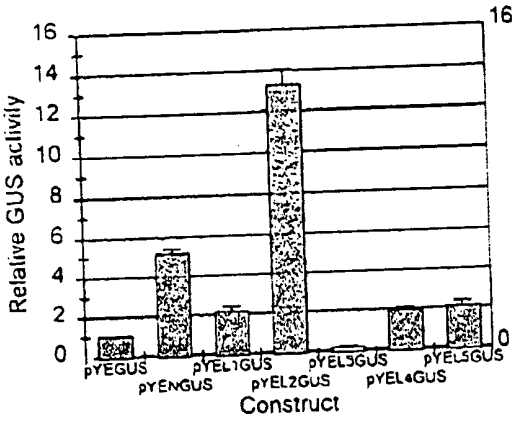


FIGURE 6C

L2	AUCUCUCAA**ACUCUCU**CGAACCUU
L2C	AUCUCUCAAACUCUCU
L2R	ACUCUCUCGAACCUU

FIGURE 6D

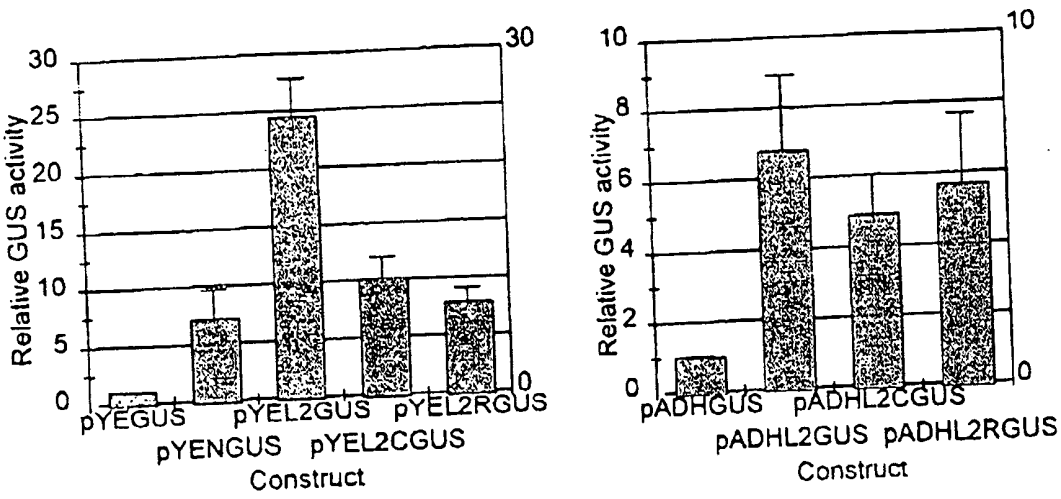


FIGURE 6E

L2	A TCT CTC AAA CTC TCT CGA ACC TT
SCAN1	a **AGA** ctc aaa ctc tct cga acc tt
SCAN2	a tct **GAG** aaa ctc tct cga acc tt
SCAN3	a tct ctc **GGG** ctc tct cga acc tt
SCAN4	a tct ctc aaa **GAG** tct cga acc tt
SCAN5	a tct ctc aaa ctc **AGA** cga acc tt
SCAN6	a tct ctc aaa ctc tct **GCT** acc tt
SCAN7	a tct ctc aaa ctc tct cga **GAG** tt

FIGURE 6F

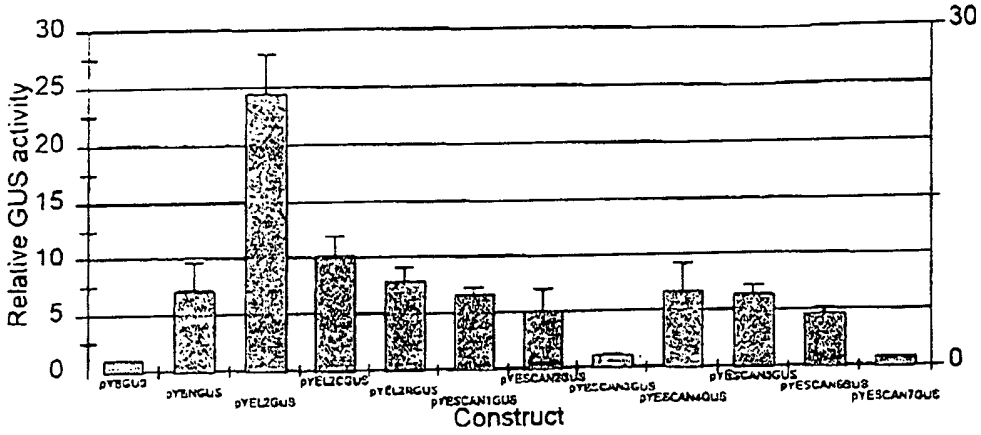


FIGURE 6G

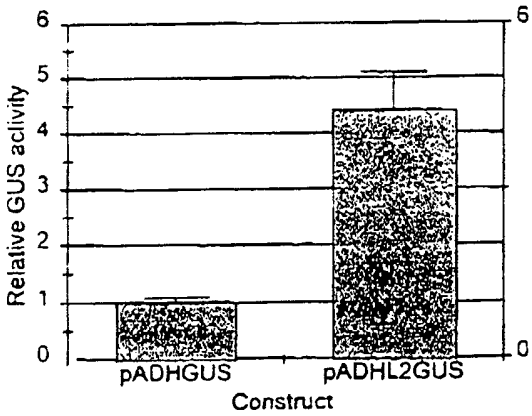
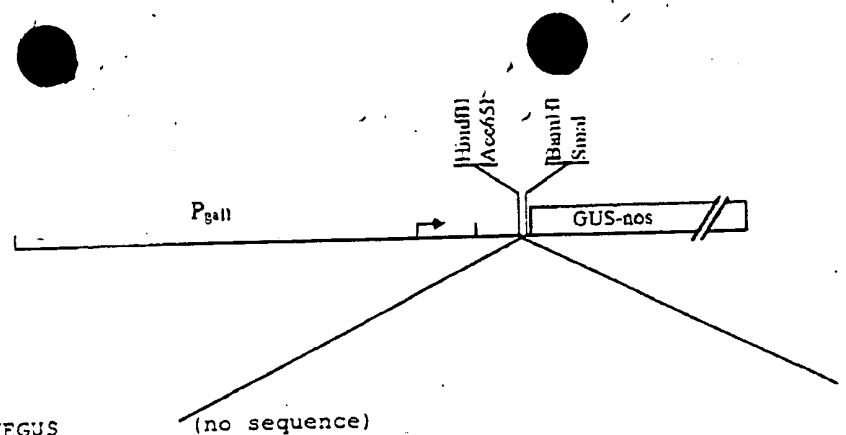


FIGURE 6H



pYEGUS	(no sequence)
pYEL1GUS	GGATCTATCCTCTTATCTCTCAA
pYEL2GUS	ATCTCTCAAACTCTCTCGAACCTT
pYEL3GUS	TTCCCCTAACCCTAGCAG
pYEL4GUS	ATCATCCTCACCTCAAAACCCACC
pYEL5GUS	AGCCTCTCATCATCCTCACCTCAA
pYEL2CGUS	ATCTCTCAAACTCTCT.
pYEL2RGUS	ACTCTCTCGAACCTT.
pYELMGUS	ACTCTCTC
pYESCAN1GUS	AAGACTCAAACTCTCTCGAACCTT
pYESCAN2GUS	ATCTGAGAAACTCTCTCGAACCTT
pYESCAN3GUS	ATCTCTCGGGCTCTCTCGAACCTT
pYESCAN4GUS	ATCTCTCAAAGAGTCTCGAACCTT
pYESCAN5GUS	ATCTCTCAAACTCAGACGAACCTT
pYESCAN6GUS	ATCTCTCAAACTCTCTGCTACCTT
pYESCAN7GUS	ATCTCTCAAACTCTCTCGAGAGTT
pYEB1-L2GUS	ATCTCTCAAACTATCTCGAAACTT
pYEB7-L2GUS	ATCTCTCAAACTCTCTCAAACTTT
pYEL2D1GUS	ATCTCTC---CTCTCTCGAACCTT
pYEL2D2GUS	ATCTCTCAAACTCTCTCGA---TT
pYEL2D3GUS	ATCTCTC---CTCTCTCGA---TT
pYE2L2GUS	ATCTCTCAAACTCTCTCGAACCTTTCTCTCAAACTCTCTCGAACCTT

LEGEND:

Vector sequence

GUS reporter gene

Start of transcription

FIGURE 6I

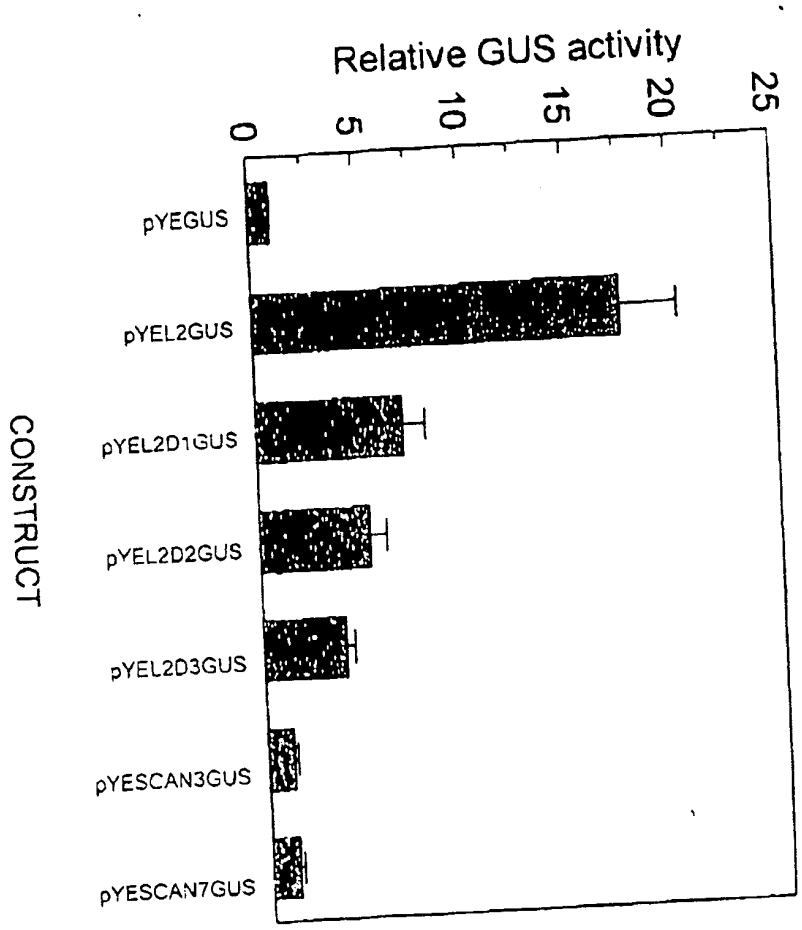

Relative GUS activity
0
5
10
15
20
25
CONSTRUCT
pYEGUS
pYEL2GUS
pYEL2D1GUS
pYEL2D2GUS
pYEL2D3GUS
pYESCAN3GUS
pYESCAN7GUS

FIGURE 6J

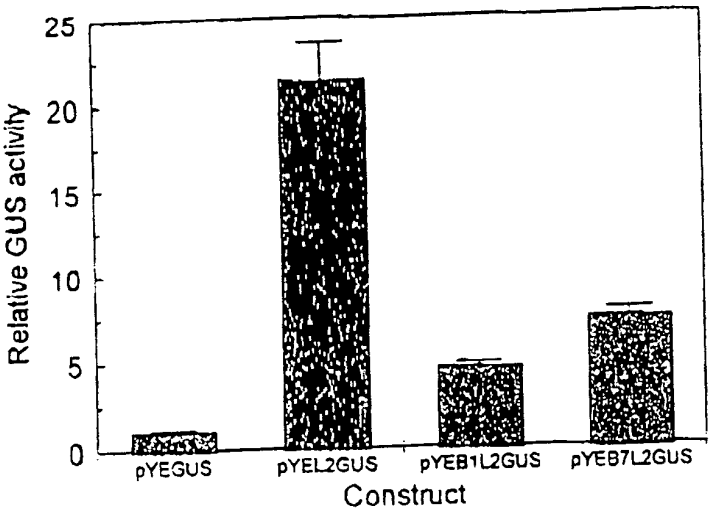

Relative GUS activity
25
20
15
10
5
0
pYEGUS
pYEL2GUS
pYEB1L2GUS
pYEB7L2GUS
Construct

FIGURE 6K

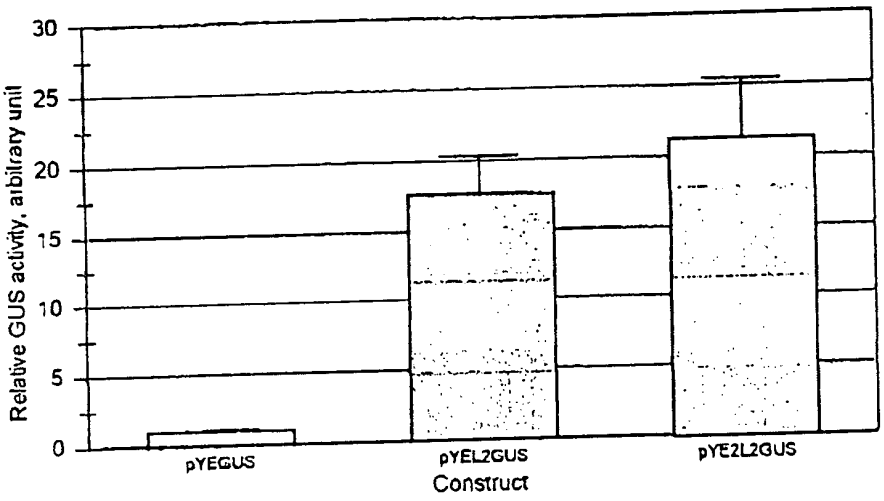
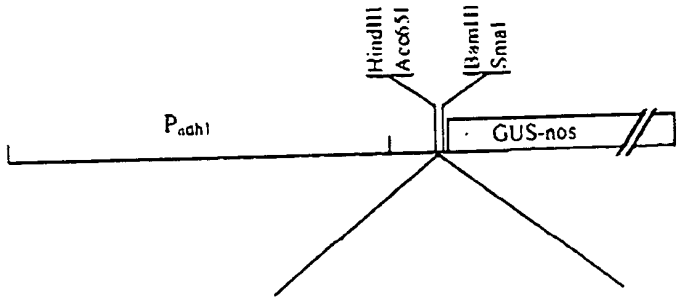

30
25
20
15
10
5
0
Relative GUS activity, arbitrary unit
pYEGUS
pYEL2GUS
pYE2L2GUS
Construct

FIGURE 6L



pADHGUS (no sequence)
pADHL2GUS ATCTCTCAAACTCTCTCGAACCTT

LEGEND:

Vector sequence

GUS reporter gene

FIGURE 6M

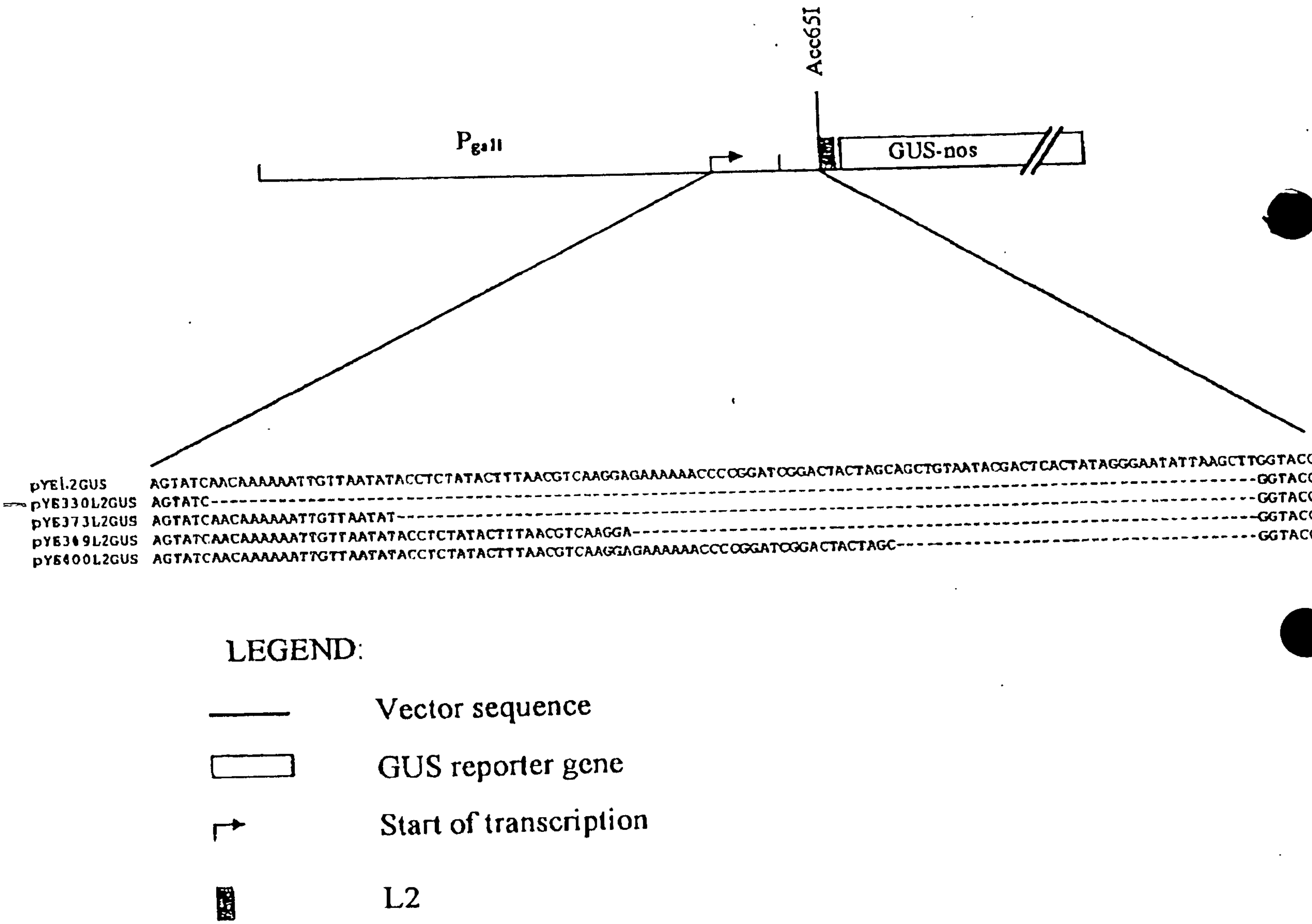

Acc65I
Pgal1
GUS-nos
pYEL2GUS AGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATCGGACTACTAGCAGCTGTAATACGACTCACTATAGGGAATATTAAGCTTGGTACC
pYE330L2GUS AGTATC------GGTACC
pYE373L2GUS AGTATCAACAAAAAATTGTTAATAT------GGTACC
pYE369L2GUS AGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGA------GGTACC
pYE400L2GUS AGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAAAACCCCGGATCGGACTACTAGC------GGTACC
LEGEND:
Vector sequence
GUS reporter gene
Start of transcription
L2

FIGURE 6N

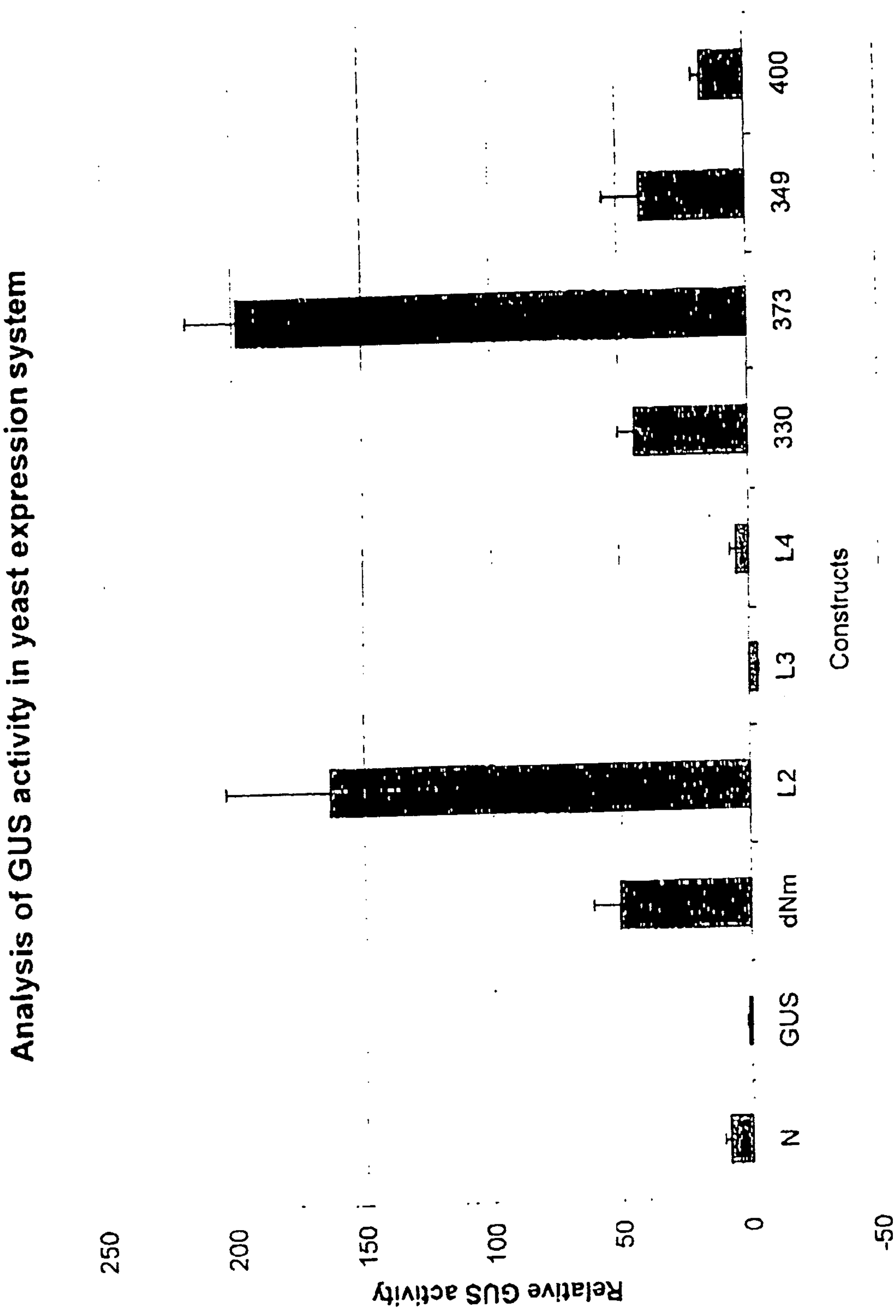

Analysis of GUS activity in yeast expression system
Relative GUS activity
250
200
150
100
50
0
-50
N
GUS
dNm
L2
L3
L4
330
373
349
400
Constructs

FIGURE 60

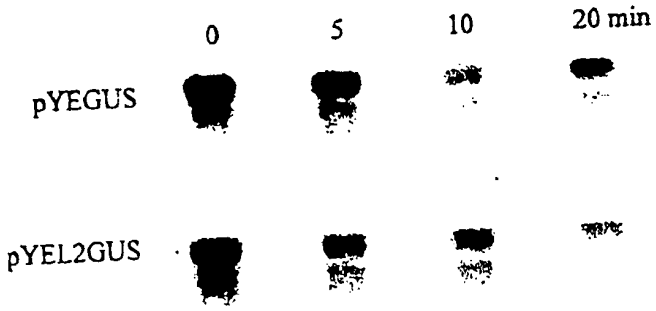


FIGURE 6P.1

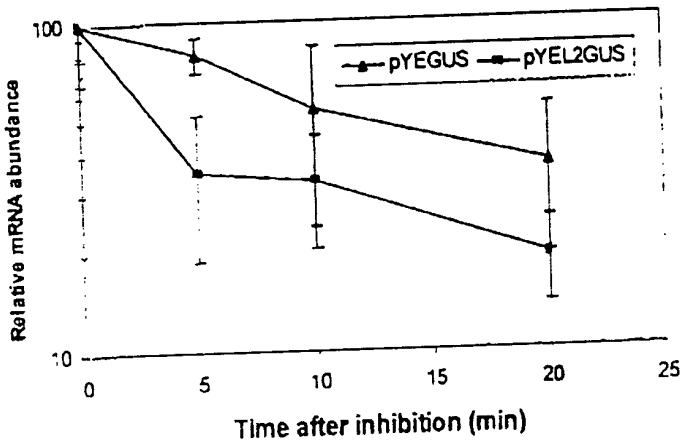

100
10
Relative mRNA abundance
pYEGUS
pYEL2GUS
0
5
10
15
20
25
Time after inhibition (min)

FIGURE 6P.2

Enhanced tCUP Versions 1-3

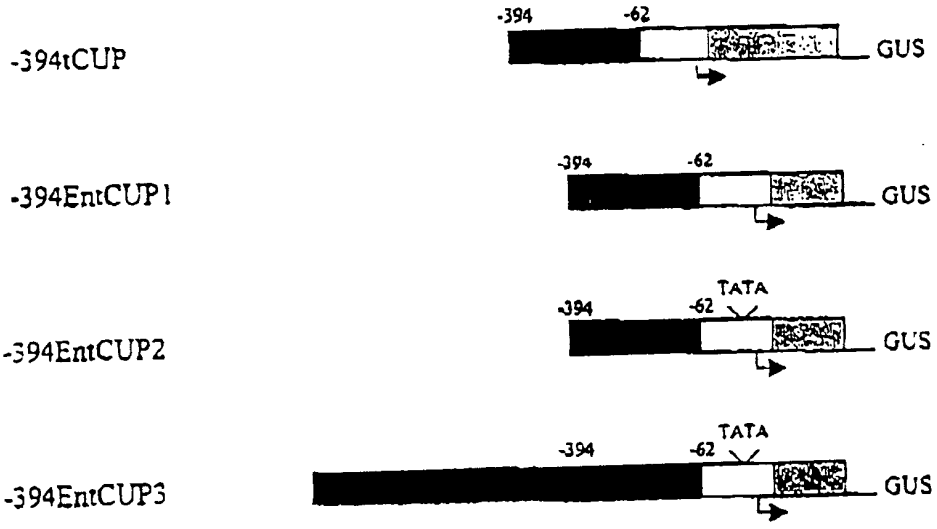
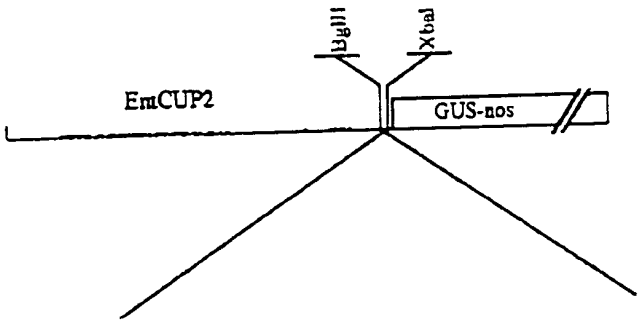


FIGURE 7A.1



pUCtCUP2(-N)GUS	(no sequence)
pUCtCUP2L1GUS	GGATCTATCCTCTTATCTCTCAA
pUCtCUP2L2GUS	ATCTCTCAAACTCTCTCGAAGCTT
pUCtCUP2L3GUS	TTCCCCTAACCCTAGCAG
pUCtCUP2L4GUS	ATCATCCTCACCTCAAAACCCACC
pUCtCUP2L5GUS	AGCCTCTCATCATCCTCACCTCAA
pUCtCUP2SCAN3GUS	ATCTCTCGGGCTCTCTCGAACCTT
pUCtCUP2SCAN7GUS	ATCTCTCAAACTCTCTCGAGAGTT
pUCtCUP2-2XL2GUS	ATCTCTCAAACTCTCTCGAACCTTTCTCTCAAACTCTCTCGAACCTT

FIGURE 7A.2

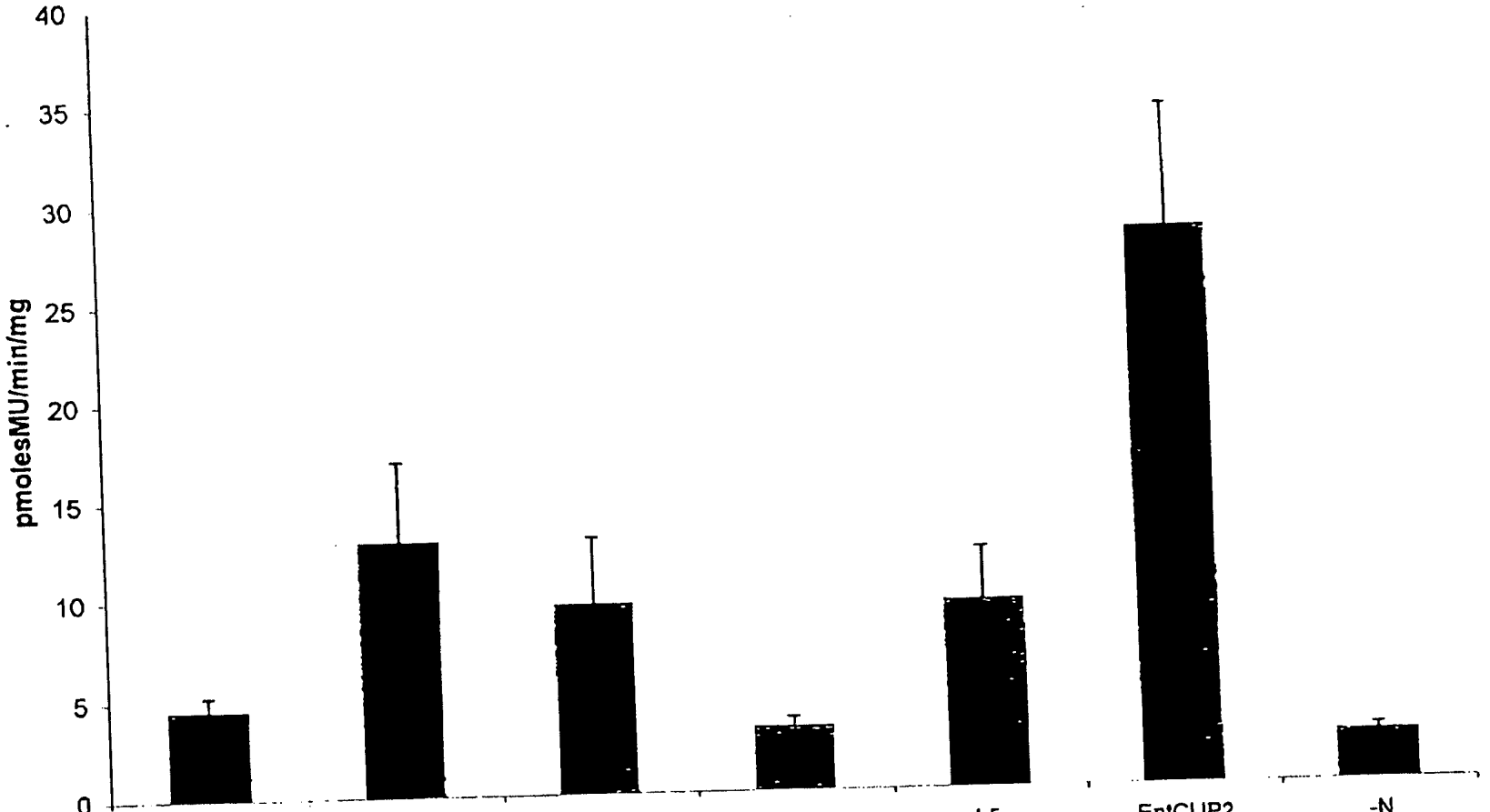


FIGURE 7B

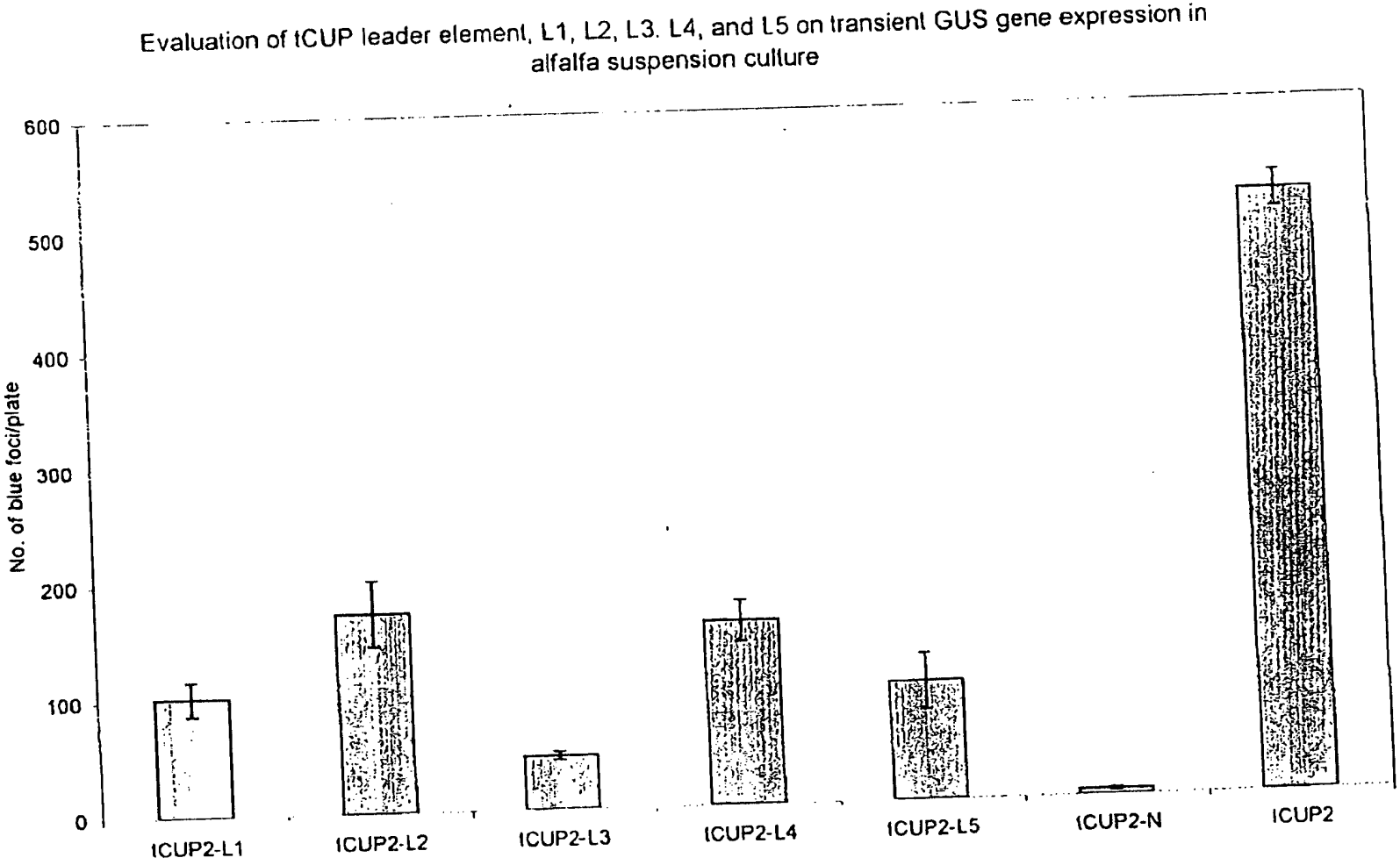


FIGURE 7C

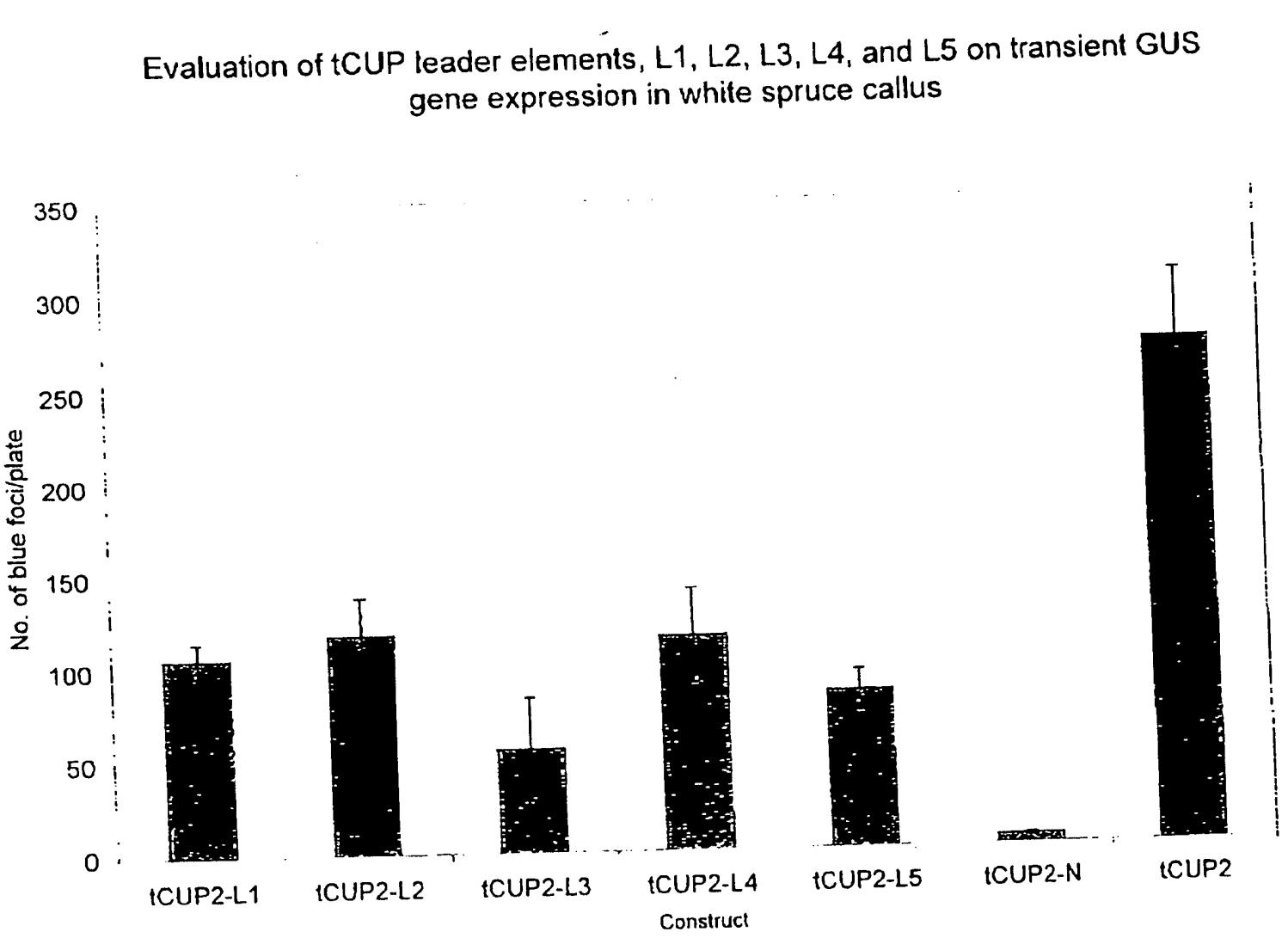
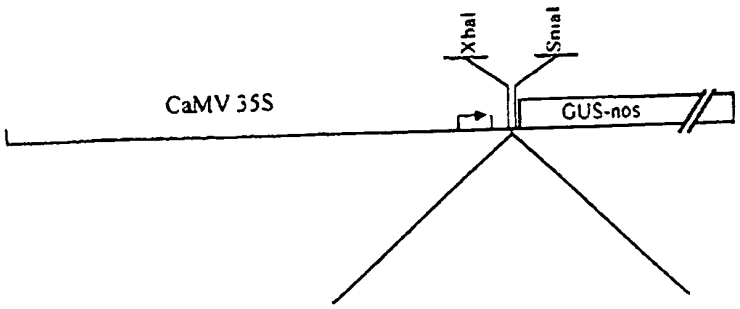

Evaluation of tCUP leader elements, L1, L2, L3, L4, and L5 on transient GUS gene expression in white spruce callus
No. of blue foci/plate
350
300
250
200
150
100
50
0
tCUP2-L1
tCUP2-L2
tCUP2-L3
tCUP2-L4
tCUP2-L5
tCUP2-N
tCUP2
Construct

FIGURE 7D



pUC35SGUS	(no sequence)
pUC35SNGUS	N
pUC35SdNmGUS	dNm
pUC35SL2GUS	ATCTCTCAAACTCTCTCGAACCTT
pUC35SL4GUS	ATCATCCTCACCTCAAAACCCACC

LEGEND:

Vector sequence

GUS reporter gene

Start of transcription

FIGURE 8A

Stable Transformation of *Arabidopsis* with GUS enhanced by L-series constructs and the 35S promoter

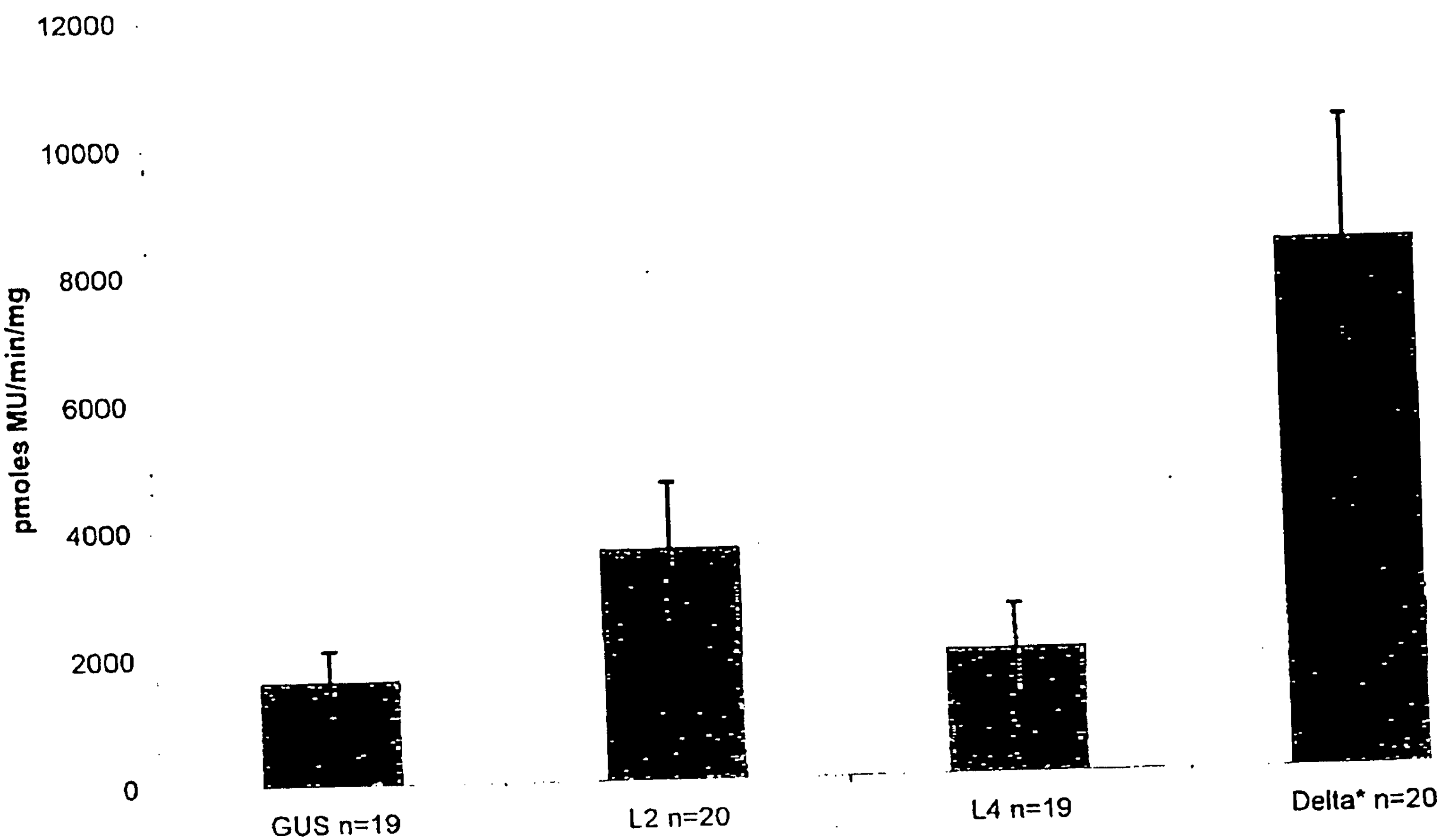


FIGURE 8B

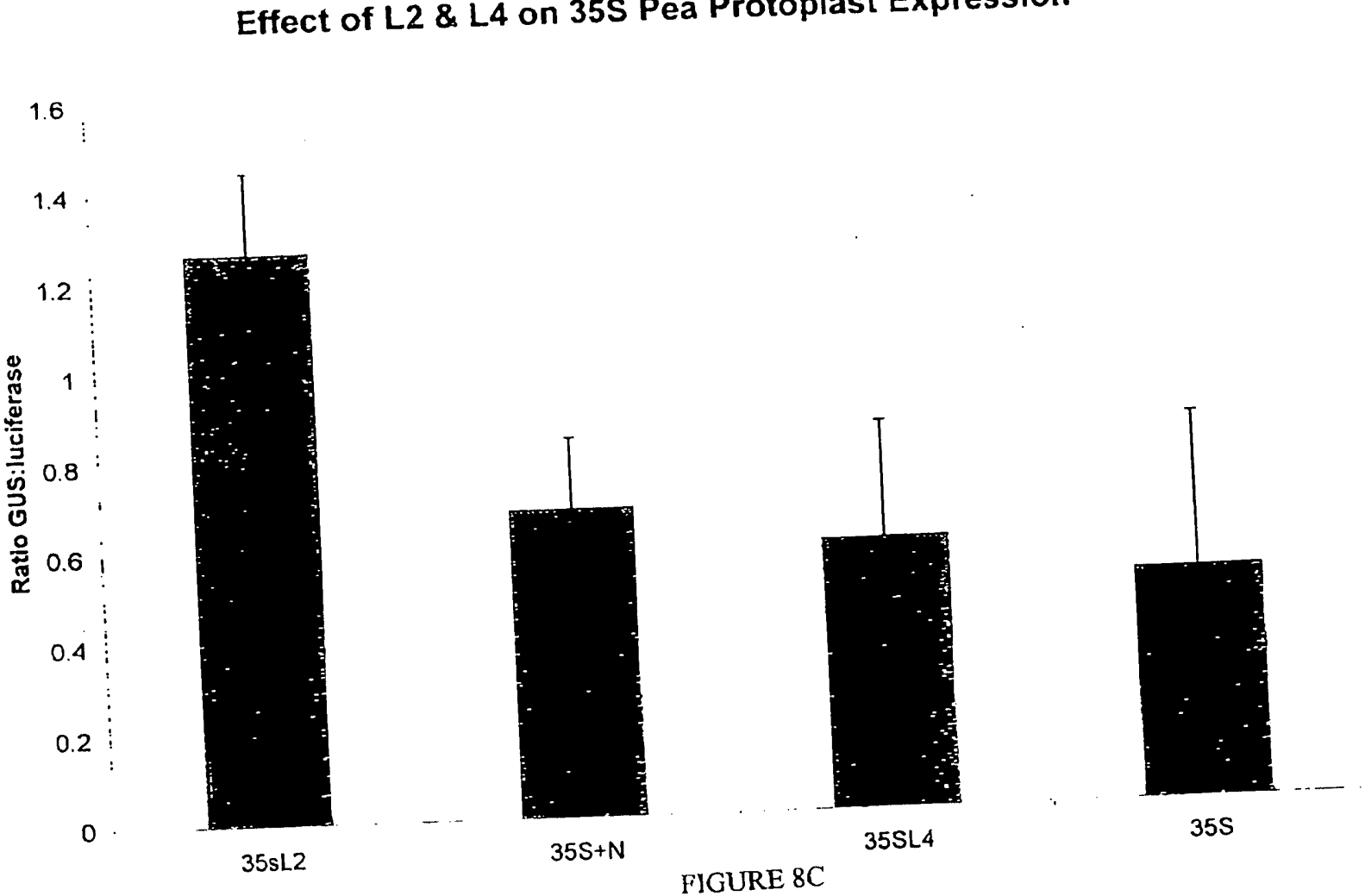

Effect of L2 & L4 on 35S Pea Protoplast Expression
Ratio GUS:luciferase
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
35sL2
35S+N
35SL4
35S

FIGURE 8C

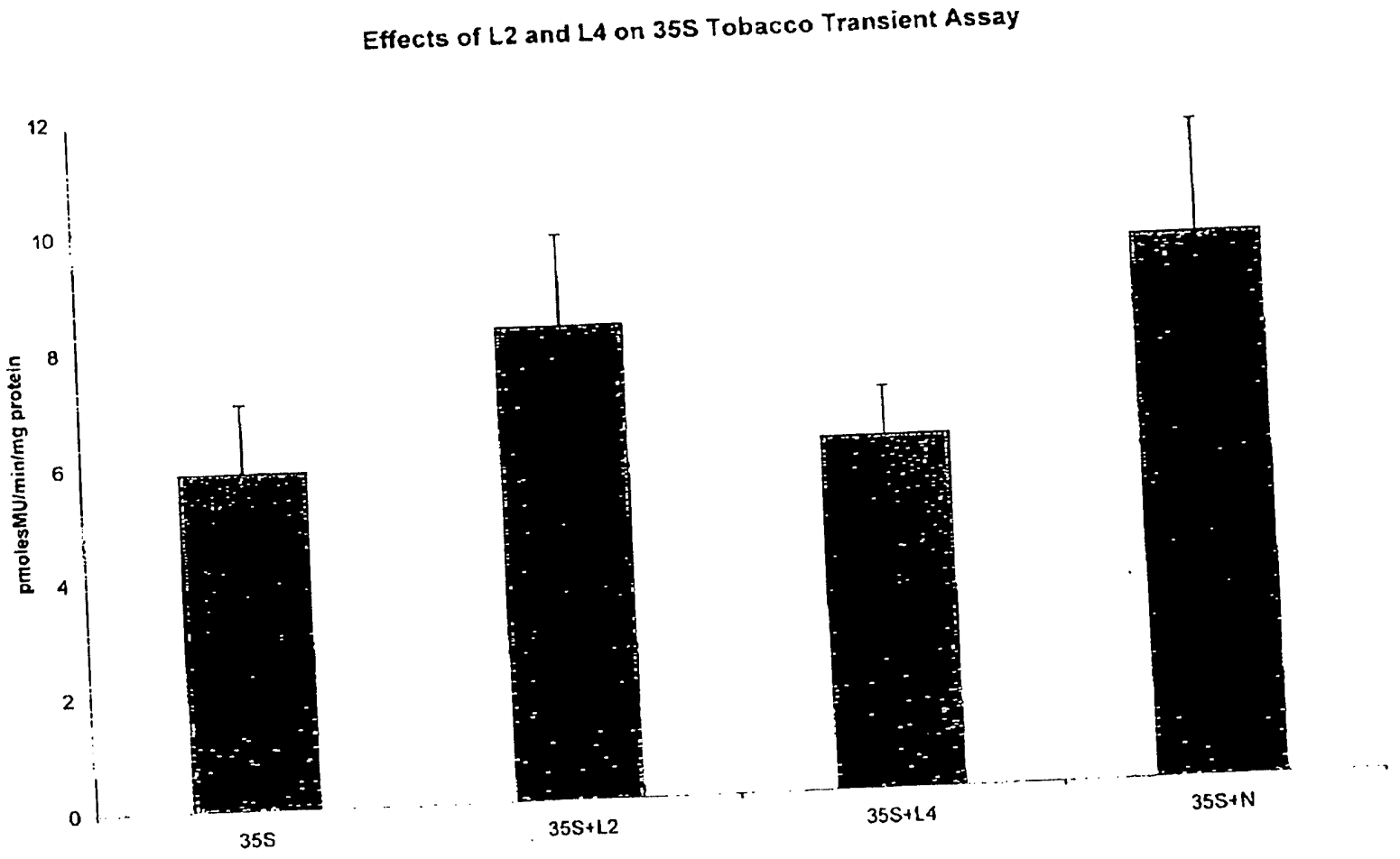


FIGURE 8D

Evaluation of the expression of tCUP leader and the elements, L2 and L4, with a heterogous promoter (35S) in a transient GUS gene expression in alfalfa suspension culture

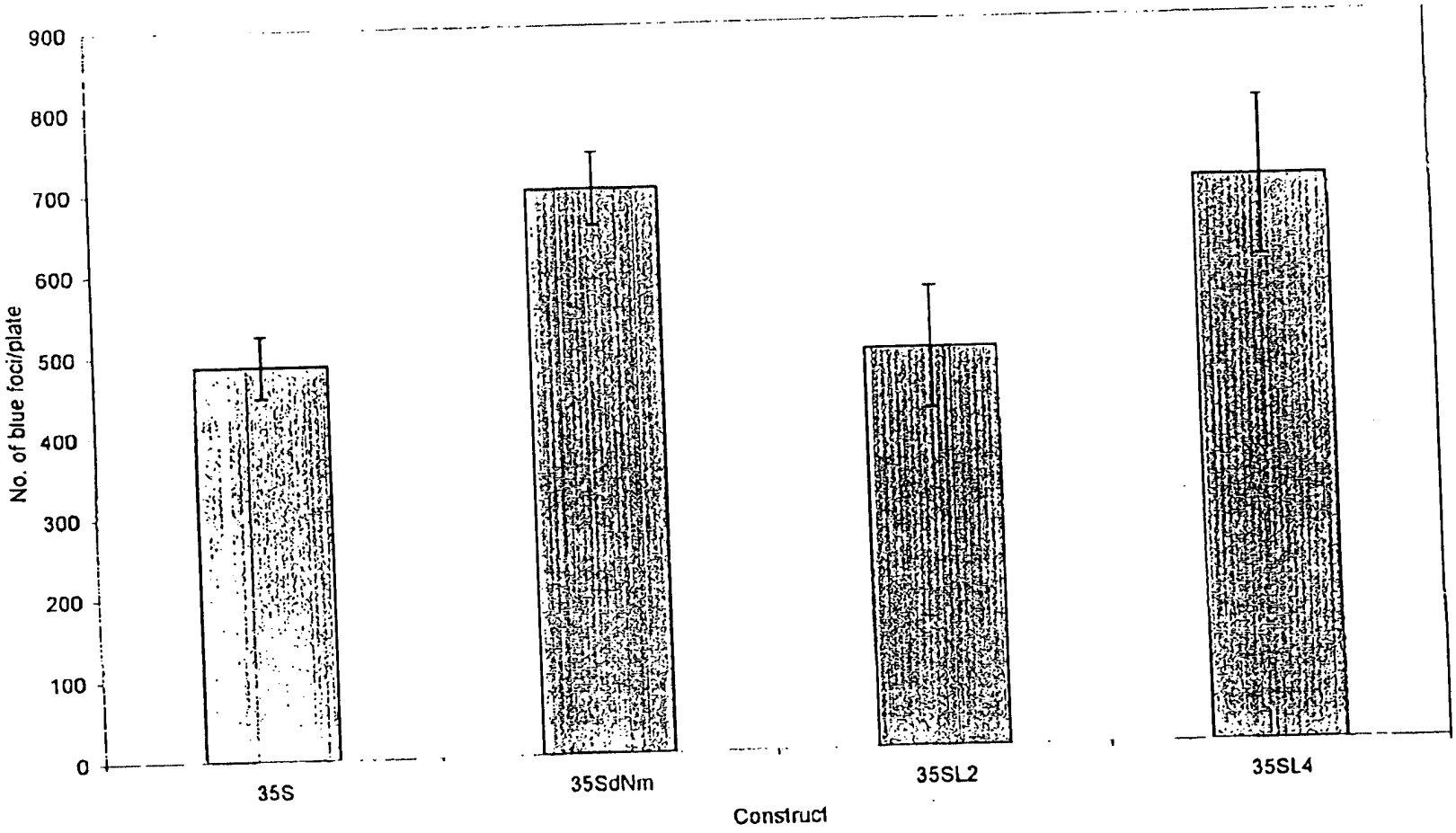
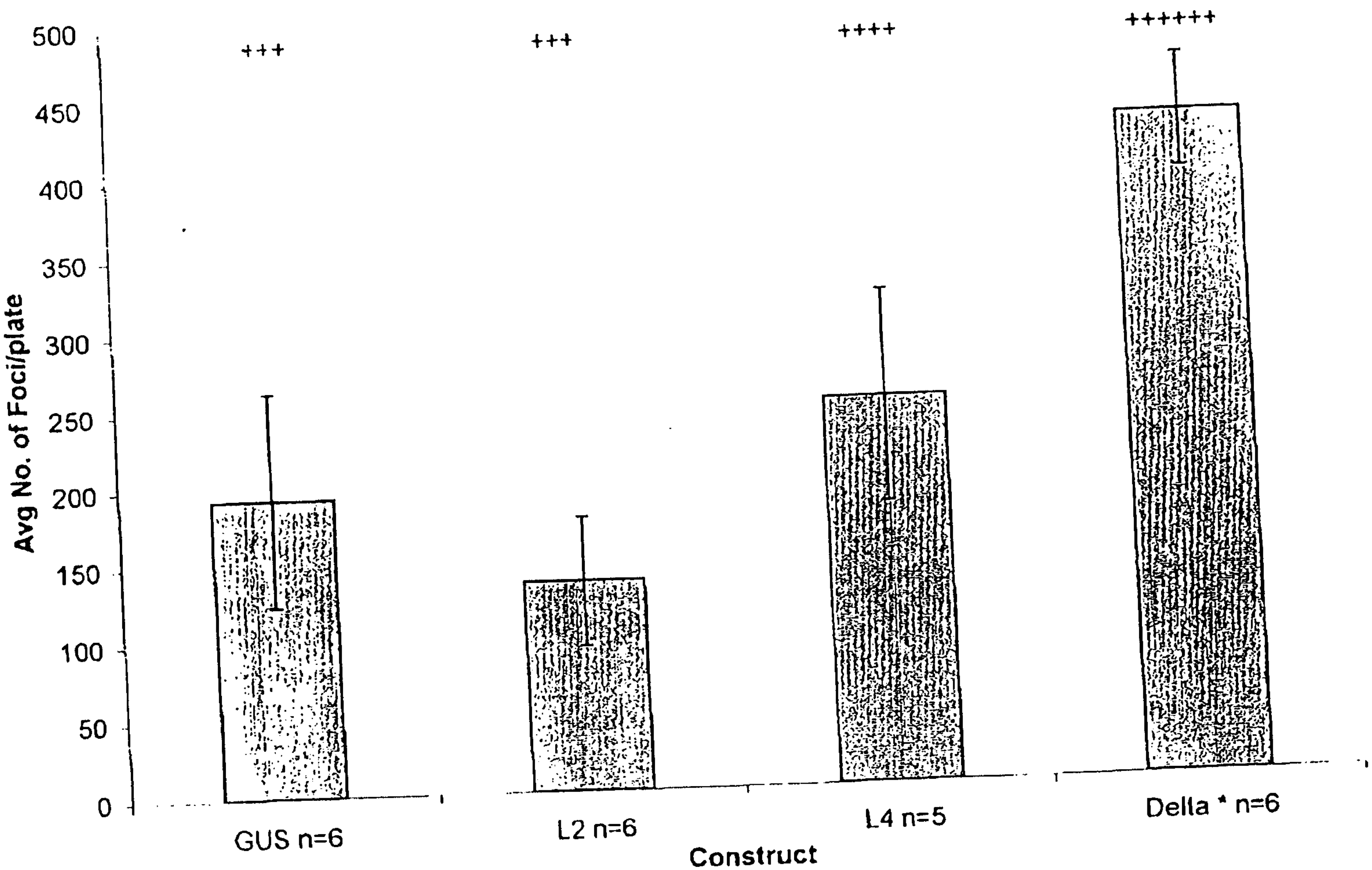


FIGURE 8E



500
450
400
350
300
250
200
150
100
50
0
Avg No. of Foci/plate
+++
+++
++++
++++++
GUS n=6
L2 n=6
L4 n=5
Della * n=6
Construct

FIGURE 8F

Evaluation of the expression of tCUP leader and the elements, L2 and L4, with a heterogous promoter (35S) in a transient GUS gene expression in white spruce callus

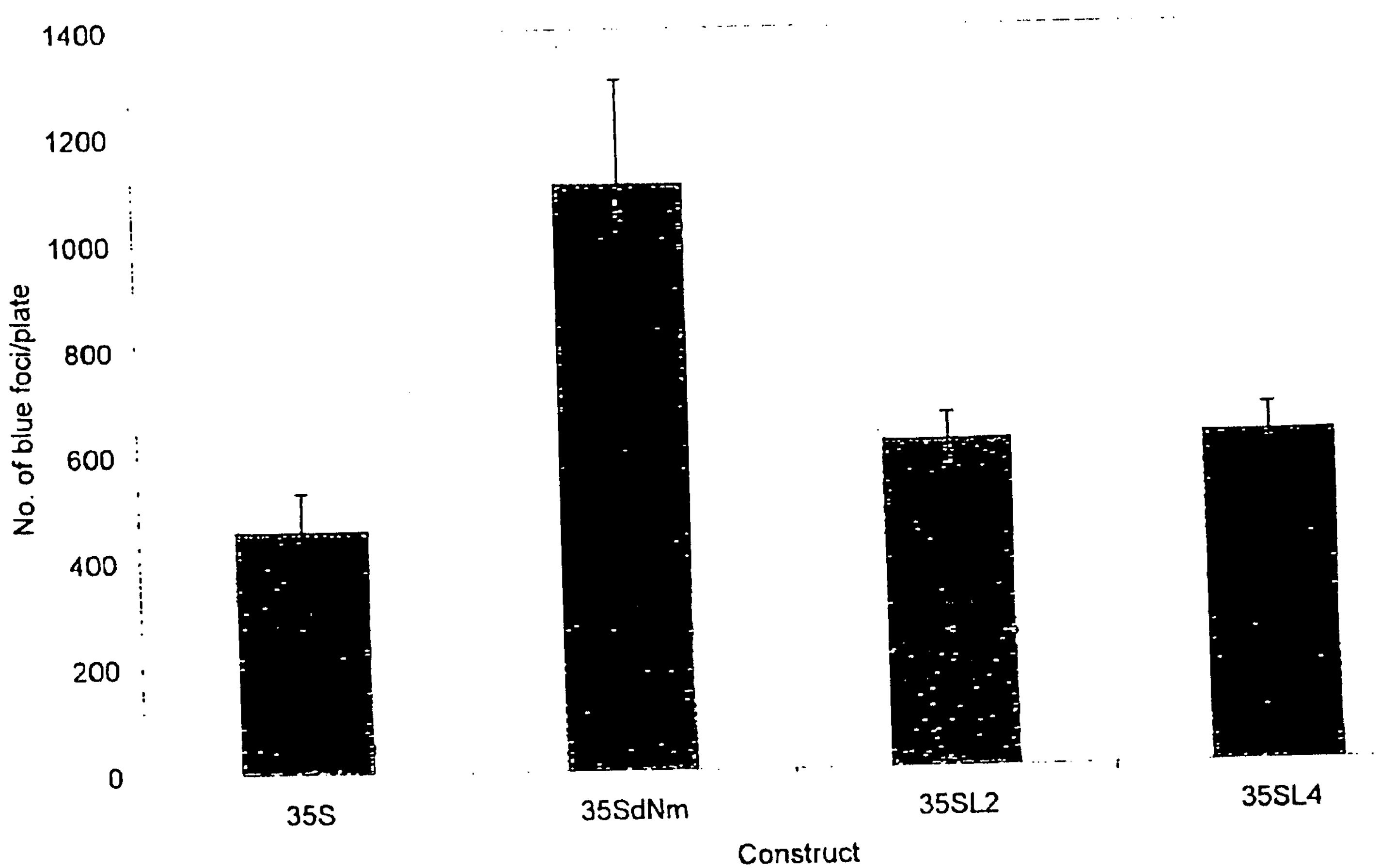
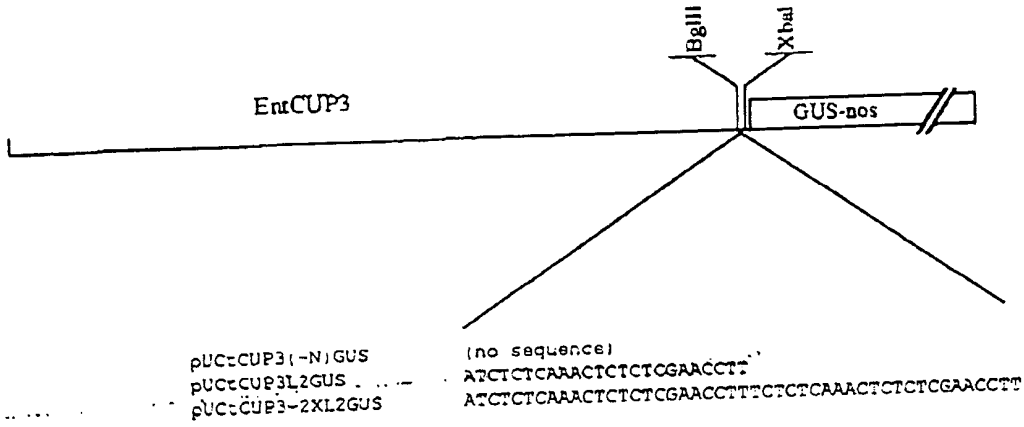


FIGURE 8G



LEGEND:

Vector sequence

GUS reporter gene

FIGURE 9A

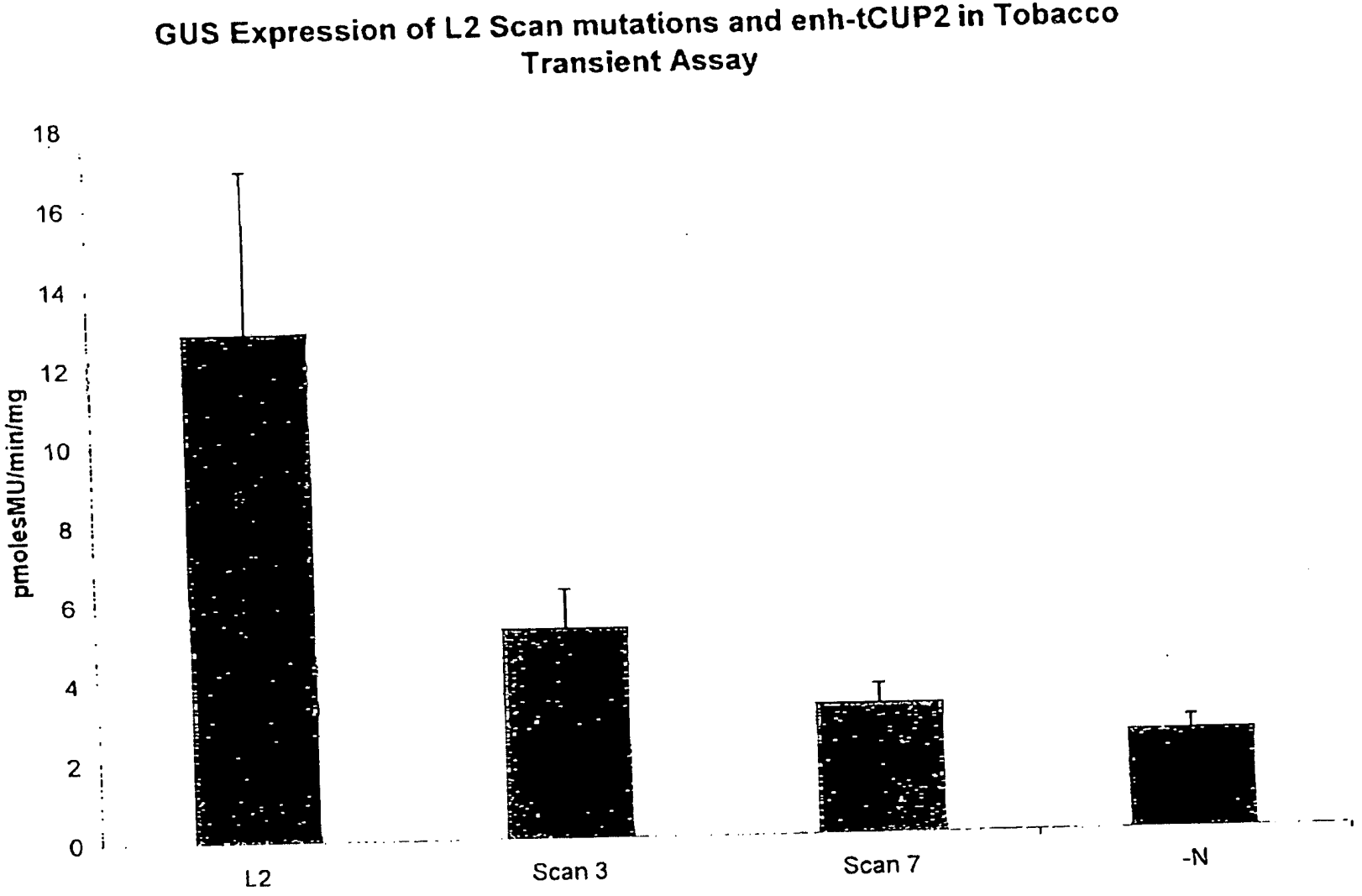

GUS Expression of L2 Scan mutations and enh-tCUP2 in Tobacco Transient Assay
pmolesMU/min/mg
18
16
14
12
10
8
6
4
2
0
L2
Scan 3
Scan 7
-N

FIGURE 9B

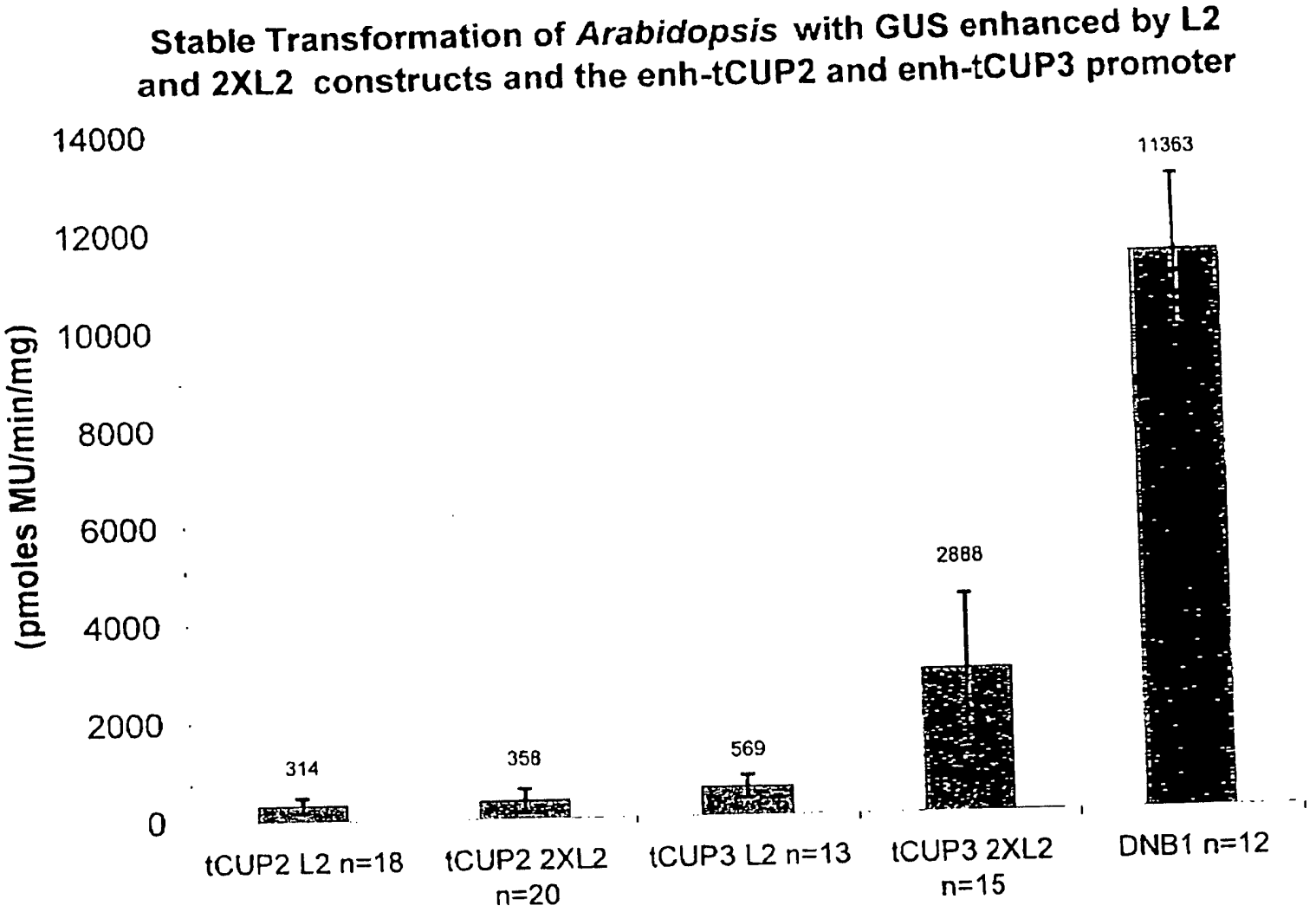


FIGURE 9C

Tobacco leaf bombardment of Enhanced tCUP vectors with L2

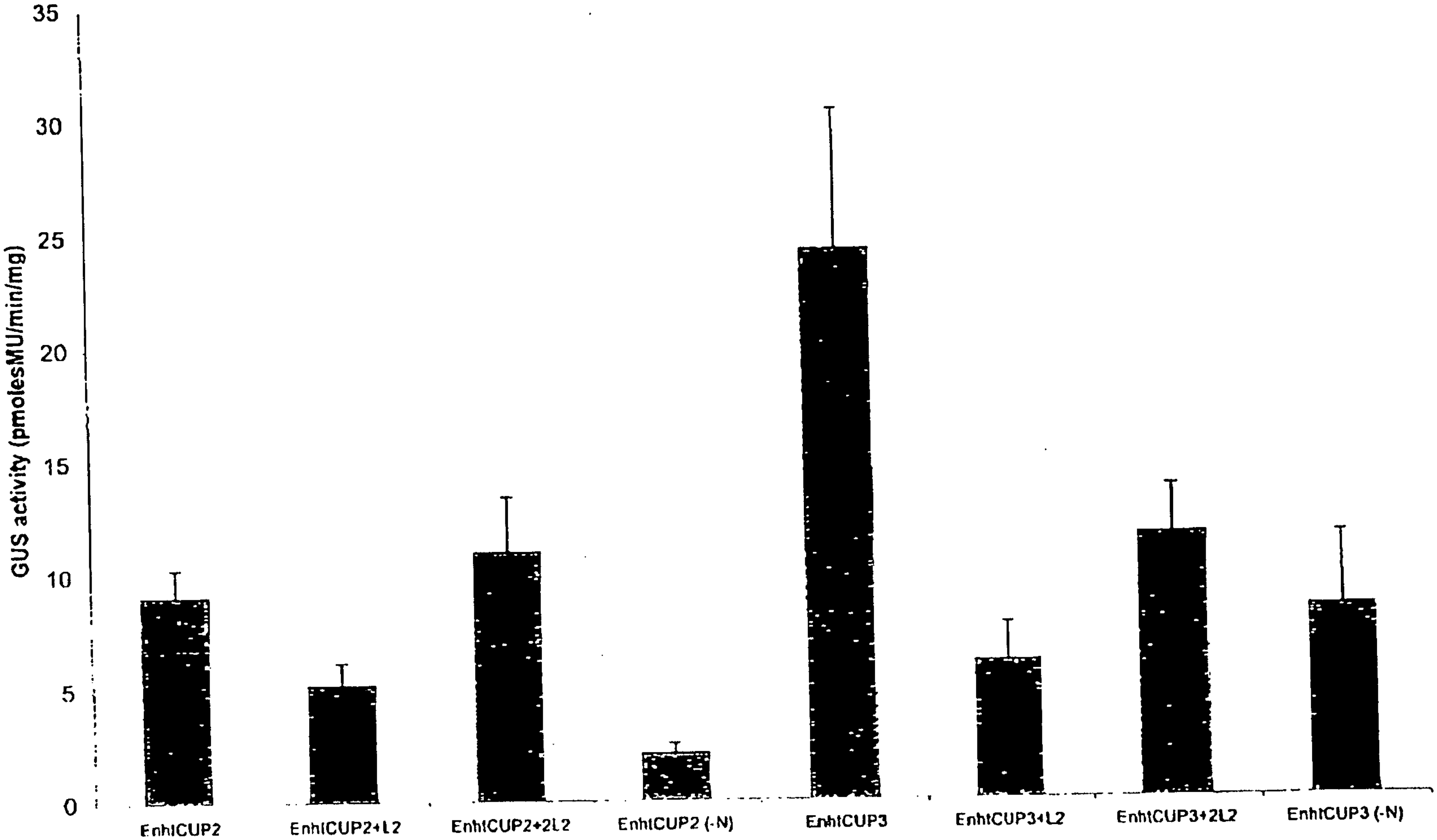


FIGURE 9D

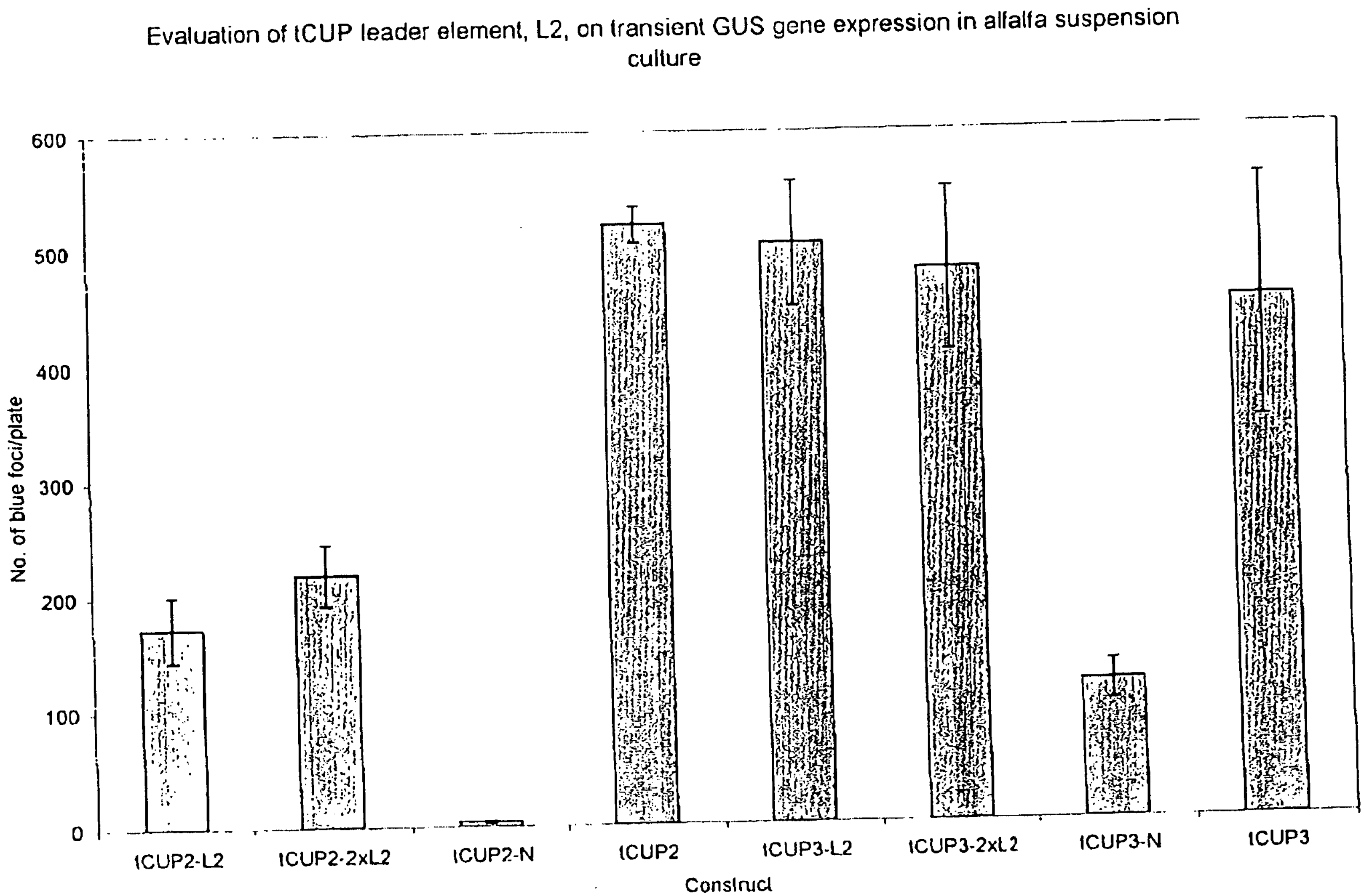


FIGURE 9E

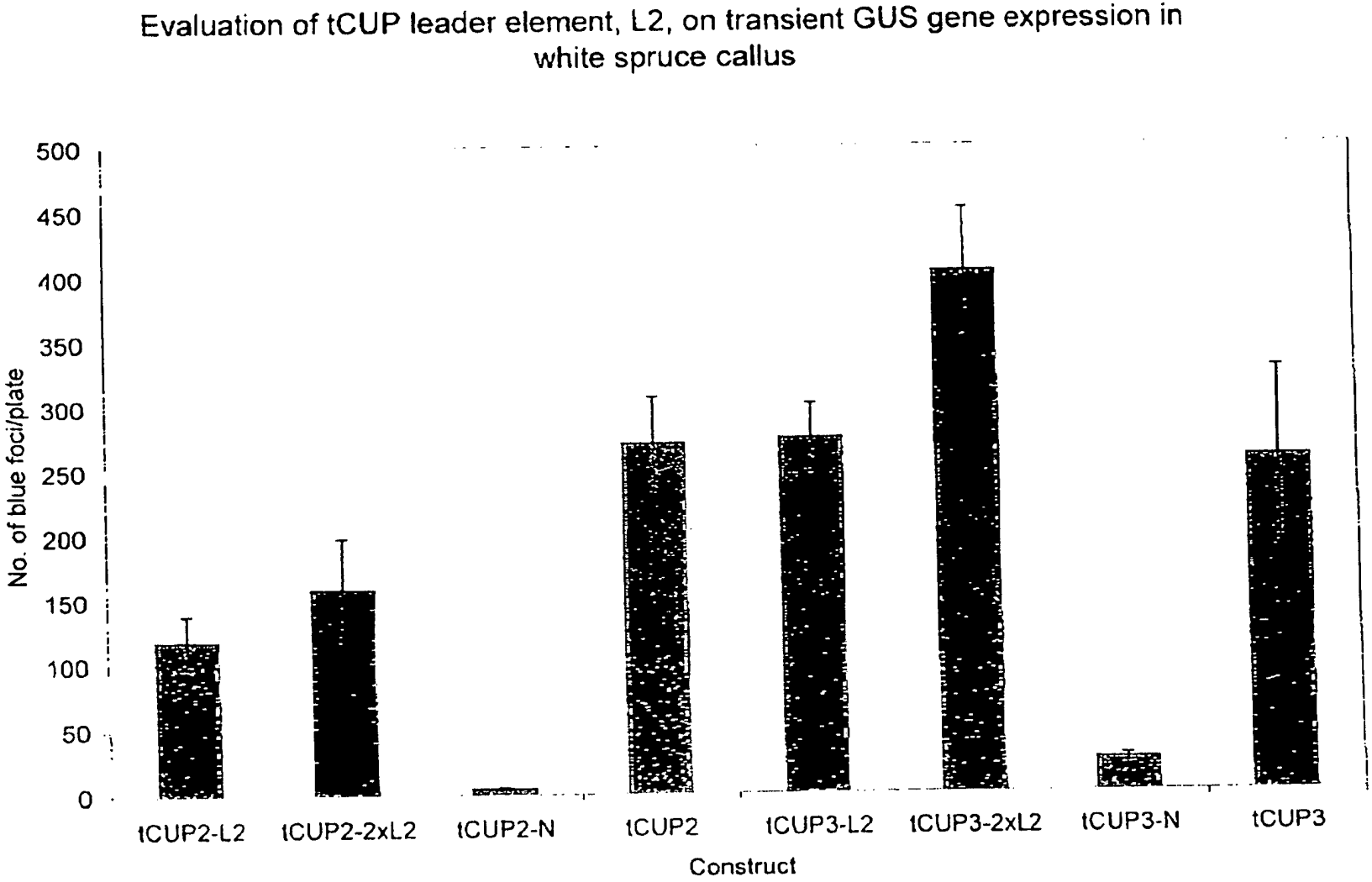
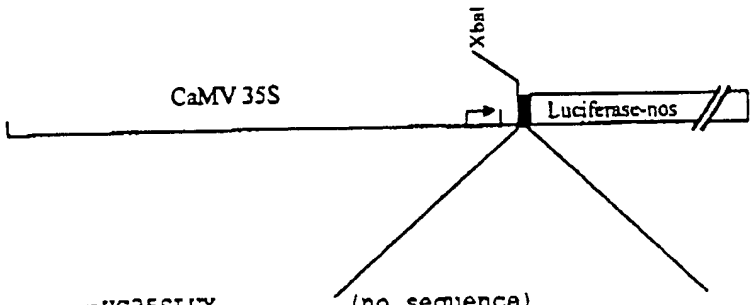


FIGURE 9F



pUC35SLUX	(no sequence)
pUC35SL2LUX	ATCTCTCAAACTCTCTCGAACCTT
pUC35SL4LUX	ATCATCCTCACCTCAAAACCCACC

LEGEND:

———	Vector sequence
▭	Luciferase reporter gene
▮	L2 or L4
↱	Start of transcription

FIGURE 10A

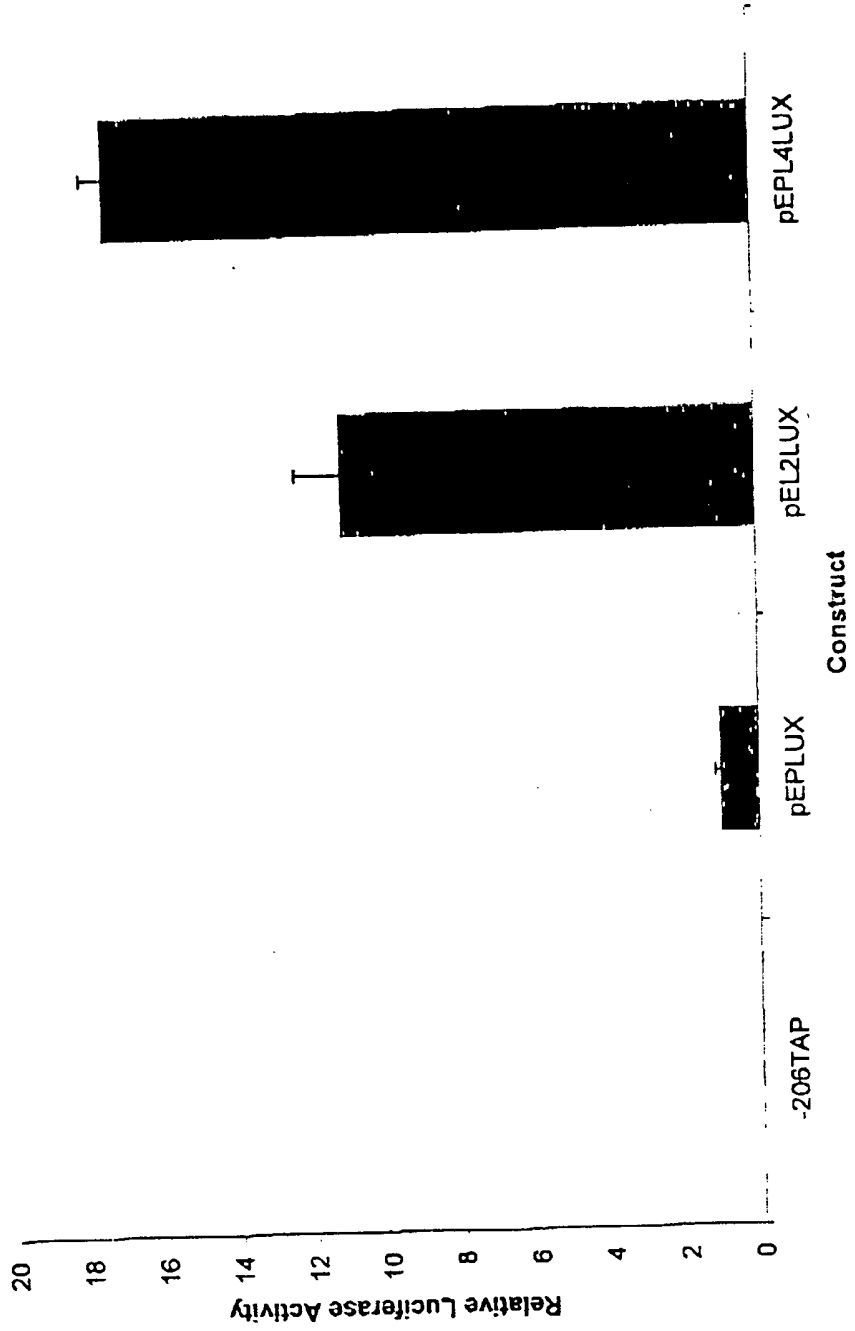

Analysis of L2 and L4 in E. Coli using a luciferase reporter system
Relative Luciferase Activity
0
2
4
6
8
10
12
14
16
18
20
-206TAP
pEPLUX
pEL2LUX
pEPL4LUX
Construct

FIGURE 10B

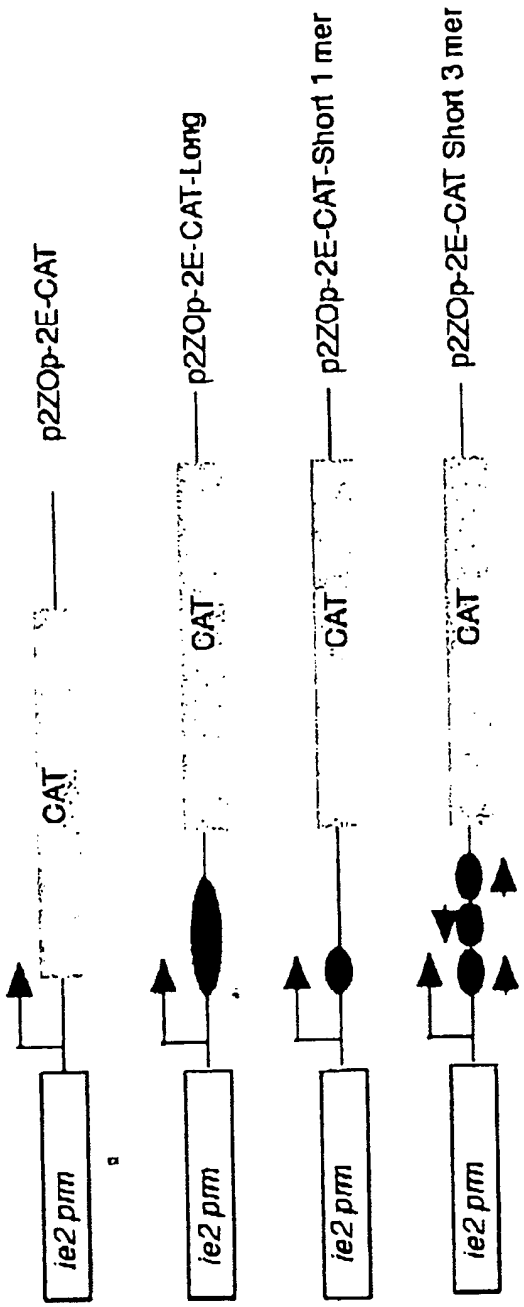

p2ZOp-2E-CAT
p2ZOp-2E-CAT-Long
p2ZOp-2E-CAT-Short 1 mer
p2ZOp-2E-CAT Short 3 mer
CAT
CAT
CAT
CAT
ie2 prm
ie2 prm
ie2 prm
ie2 prm

FIGURE 11A

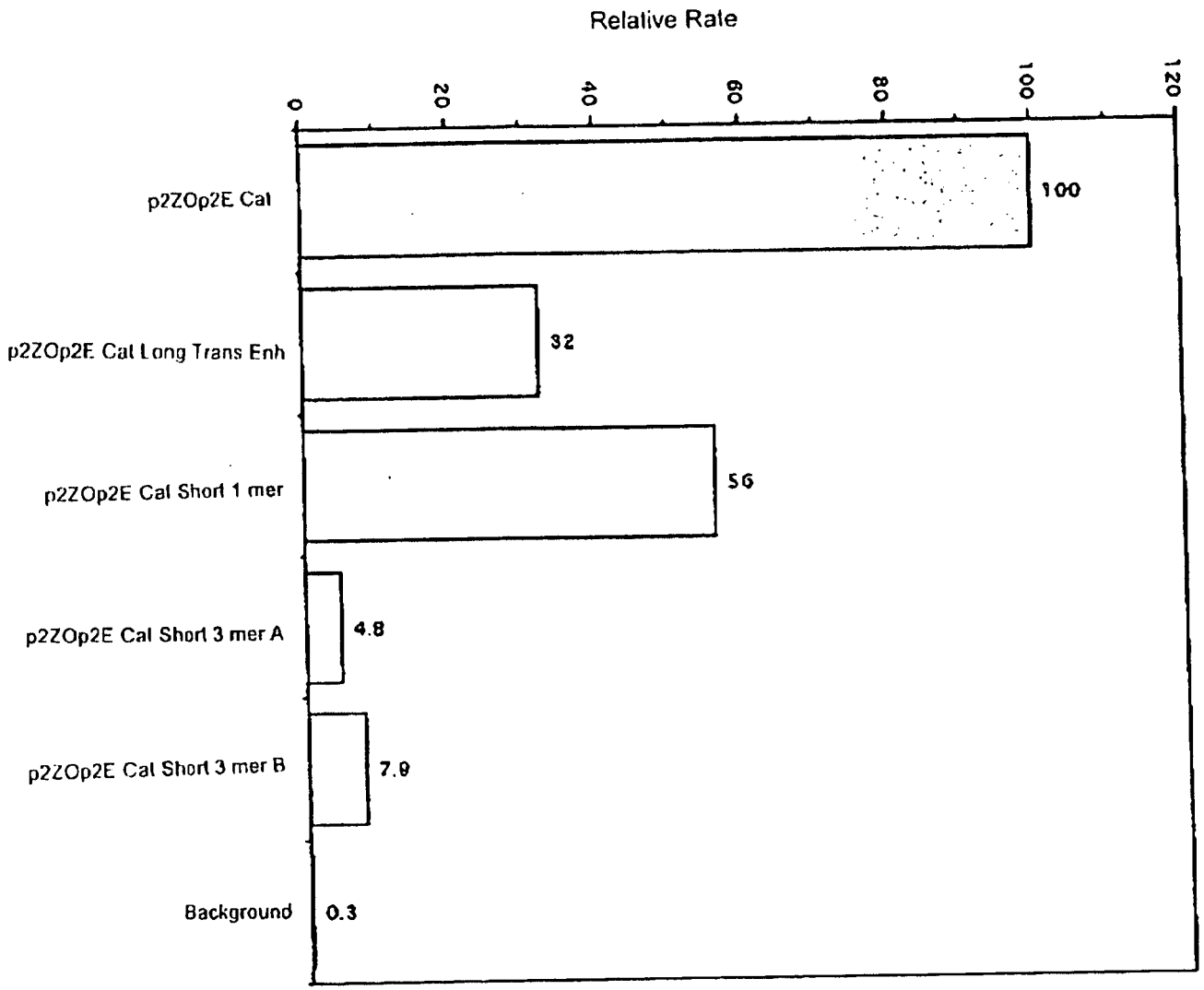

Relative Rate
0
20
40
60
80
100
120
p2ZOp2E Cat
100
p2ZOp2E Cat Long Trans Enh
32
p2ZOp2E Cat Short 1 mer
56
p2ZOp2E Cat Short 3 mer A
4.8
p2ZOp2E Cat Short 3 mer B
7.9
Background
0.3

FIGURE 11B